

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
REQUEST FOR FILING APPLICATION UNDER 37 CFR 53(b)  
WITHOUT FILING FEE OR EXECUTED INVENTOR'S DECLARATION

Assistant Commissioner for Patents  
Washington, D.C. 20231

Atty. Dkt. 1579-315  
Date: **November 10, 1999**

This is a request for filing a new PATENT APPLICATION under Rule 53(b) entitled:

**STRESSOR REGULATED GENES**

without a filing fee and/or without an executed inventor's oath/declaration.

This application is made by the below identified inventor(s). Attached hereto are the following papers:

☒ An abstract together with

☒ 34 pages of specification and claims including

☒ 4 numbered claims and also attached is/are

☒ 43 sheets of accompanying drawings.

☐ This application is based on the following prior foreign application(s):

**Application No.**

**Country**

**Filing Date**

respectively, the entire content of which is hereby incorporated by reference in this application, and priority is hereby claimed therefrom.

☒ This application is based on the following prior provisional application(s):

**Application No.**

**Filing Date**

**60/109,281**

**November 20, 1998**

respectively, the entire content of which is hereby incorporated by reference in this application, and priority is hereby claimed therefrom.

☐ Certified copy/ies of foreign applications attached.

This application is a ☐ continuation/☐ division/☐ continuation-in-part of application Serial No. , filed

Please amend the specification by inserting before the first line: --This application is a ☐ continuation/☐ division/☐ continuation-in-part of application Serial No. , filed , the entire content of which is hereby incorporated by reference in this application.--

☐ Please amend the specification by inserting before the first line: --This is a continuation of PCT application No. , filed , the entire content of which is hereby incorporated by reference in this application.--

☒ Please amend the specification by inserting before the first line: --This application claims the benefit of U.S. Provisional Application No. **60/109,281**, filed **November 20, 1998**, the entire content of which is hereby incorporated by reference in this application.--

☐ Preliminary amendment to claims (attached hereto), to be entered before calculation of the fee.

☐ Also attached.

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# ***U.S. PATENT APPLICATION***

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***Invention:*** STRESSOR REGULATED GENES

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## ***SPECIFICATION***

## STRESSOR REGULATED GENES

### TECHNICAL FIELD

The present invention relates, in general, to stressor-responsive genes and, in particular, to metal-responsive genes, to mRNAs, to proteins encoded therein and to uses thereof, for example, as biomonitors and in drug discovery.

### BACKGROUND

The transition metal cadmium is considered to be a serious occupational and environmental toxin. Cadmium was ranked number 7 on the Agency for Toxic Substances and Disease Registry/Environmental Protection Agency "Top 20 Hazardous Substances Priority List" in 1997 (Fay et al. (1997) *Food Chem. Toxicol.* 34, 1163-1165). In addition, it is a frequently found contaminant at Superfund sites (Fay et al. (1997) *Food Chem. Toxicol.* 34, 1163-1165). Cadmium is used primarily in metal coatings, nickel-cadmium batteries and pigments (Friberg et al. (1986) in *Handbook of the Toxicology of Metals* (Friberg, L, Nordberg G.F. and Vouk, V., ed) pp. 130-237, Elsevier/North-Holland, Amsterdam; Aylett, B.J. (1979) in *The Chemistry, Biochemistry and Biology of Cadmium* (Webb, M., ed) pp. 1, Elsevier/North-Holland, New York). It is also continuously introduced into the atmosphere through the smelting of ores and the burning of fossil fuels (Friberg et al. (1986) in *Handbook of the Toxicology of Metals* (Friberg, L, Nordberg G.F. and

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Vouk, V., ed) pp. 130-237, Elsevier/North-Holland, Amsterdam; Aylett, B.J. (1979) in *The Chemistry, Biochemistry and Biology of Cadmium* (Webb, M., ed) pp. 1, Elsevier/North-Holland, New York). It has been suggested that increased industrialization has resulted in higher levels of accumulated cadmium in humans (Fortoul et al. (1996) *Environ. Health Perspect.* 104, 630-632). The primary routes of non-occupational exposure in humans are via inhalation, and ingestion of cadmium-containing food (Waalkes et al. (1992) *Crit. Rev. Toxicol.* 22, 175-201). Humans are continuously exposed to cadmium and accumulate the metal throughout their lives in liver, lung and kidney tissue (Aylett, B.J. (1979) in *The Chemistry, Biochemistry and Biology of Cadmium* (Webb, M., ed) pp. 1, Elsevier/North-Holland, New York; Bernard et al. (1986) *Experientia Suppl.* 50, 114-123). Toxicological responses of cadmium exposure include kidney damage, respiratory diseases, such as emphysema and neurologic disorders (Waalkes et al. (1992) *Crit. Rev. Toxicol.* 22, 175-201; Chmielnicka et al. (1986) *Biol. Trace Elements Res.* 10, 243-256). Cadmium has been classified as a type 1 human carcinogen (International Agency for Research on Cancer (1993) *Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry*, Vol. 58, IARC, Lyon). It induces site of exposure, lung, kidney, prostate and testicular cancers in rats and mice (Waalkes et al. (1992) *Crit. Rev. Toxicol.* 22, 175-201). Human epidemiological data suggests that it causes tumors of the male reproductive system and induces respiratory

tumors (Waalkes et al. (1992) *Crit. Rev. Toxicol.* 22, 175-201; Oberdorster, G. (1986) *Scand. J. Work Environ. Health* 12, 523-537).

Intracellular damage associated with cadmium exposure includes protein denaturation, lipid peroxidation and DNA strand breaks. Proposed mechanisms by which cadmium induces this damage involve (a) metal binding to reduced cysteine residues and (b) the generation of reactive oxygen species, possibly by lowering reduced glutathione levels (Abe, T. et al. (1994) *Biochim. Biophys. Acta.* 1201, 29-36; Manca, D. et al. (1991) *Toxicology* 67, 303-323; Chin, T. A. et al. (1993) *Toxicology* 77, 145-156). To prevent cadmium-induced intracellular damage, cells respond to metal exposure by inducing the transcription of genes that encode defense and repair proteins. These proteins (a) chelate the metal to prevent further damage, (b) remove reactive oxygen species, (c) repair membrane and DNA damage and (d) renature or degrade unfolded-proteins. Cadmium has been shown to affect the steady-state levels of the mRNAs encoding metallothionein (Hamer, D.H. (1986) *Annu. Rev. Biochem.* 55, 913-951), heme oxygenase (Adam, J. et al. (1989) *J. Biol. Chem.* 264, 6371-6375),  $\gamma$ -glutamylcysteine synthetase (Hatcher. E.L. et al. (1995) *Free Radic. Biol. Med.* 19, 805-812), low and high molecular weight heat shock proteins (Wiegant. F.A. et al. (1994) *Toxicology* 94, 143-159) and ubiquitin (Muller-Taubenberger, A. et al. (1988) *J. Cell Sci.* 90, 51-58). In addition, increases in superoxide dismutase, catalase, glutathione peroxidase and glucose-6-phosphate

dehydrogenase activities are observed following cadmium exposure in cultured cells and whole animals (Kostic, M.M. et al. (1993). *Eur. J. Haematol.* 51, 86-92; Salovsky P. et al. (1992) *Hum. Exp. Toxicol.* 11, 217-222). The mechanism(s) by which this metal modulates the levels of expression of most of these genes remains unknown.

Cadmium-activated transcription may occur through specific metal-responsive upstream regulatory elements found in the promoters of cadmium-responsive genes. These may include metal responsive element (MRE) sequences, found in most metallothionein genes (Stuart, G.W. et al. (1984) *Proc. Natl. Acad. Sci. USA* 81, 7381-7322; Searle, P.F. (1990) *Nucleic Acids Res.* 18, 4863-4690; Cizewski Culotta, V.C. et al. (1989) *Mol. Cell. Biol.* 9, 1376-1380), or cadmium-responsive elements, as found in the human heme oxygenase gene (Takeda, K. et al. (1994) *J. Biol. Chem.* 265, 14061-14064). Cadmium may also affect gene expression by influencing signal transduction pathways. Cadmium affects the activities of PKC, PKA and calmodulin (Wang, Z. et al. (1998) *J. Biol. Chem.* 273, 73-79; Beyersmann, D. et al. (1997) *Toxicol. Appl. Pharmacol.* 144, 247-261). It has been suggested that cadmium-induced transcription of the proto-oncogenes *jun* and *fos* is mediated via PKC and calmodulin (Wang, Z. et al. (1998) *J. Biol. Chem.* 273, 73-79). Thus, cadmium can modulate the activities of complex signal transduction pathways that in turn can influence the expression of a myriad of genes. However, relatively few cadmium-responsive genes have been

identified. In addition, there is a paucity of information on the influence of cell-specific and developmental factors on metal-inducible gene expression.

#### SUMMARY OF THE INVENTION

The present invention relates to stressor-regulated genes in general and specifically to metal-regulated genes, such as cadmium-regulated genes, mRNAs and to the proteins encoded therein. The invention also relates to the use of such genes and proteins as biomonitors and in drug discovery.

Objects and advantages of the present invention will be clear from the description that follows.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**Fig. 1. Confirmation of cadmium-responsive gene expression.** Total RNA was extracted from control *C. elegans* (-) and nematodes exposed to 100 $\mu$ M CdCl<sub>2</sub> for 24h (+) and 20  $\mu$ g was then subjected to denaturing gel electrophoresis. Northern blots were hybridized with a <sup>32</sup>P-labeled oligonucleotide probe that is specific for *mtl-2* mRNA; nt 182-221 (*upper panel*) (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564). Following autoradiography, the labeled probe was removed and the membrane reprobed with a <sup>32</sup>P-labeled DNA probe specific for the myosin light-chain mRNA (*lower panel*). There are two forms of *C. elegans* myosin light-chain mRNA, containing 900 and 1300 nt (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564; Cummins, C. et al. (1988) *Mol. Cell. Biol* 8, 5334-5349).

**Figs. 2A and 2B. Representative mRNA differential display band patterns of control and cadmium-treated *C. elegans*.** Total RNA was isolated

from *C. elegans* exposed to cadmium for 0, 8 and 24 h and analyzed by differential display. RNA from duplicate populations of treated and control *C. elegans* was reverse transcribed and amplified with the 3'-degenerate anchored oligo(dT) primer T<sub>12</sub>MA and the 5'-arbitrary decamer AP-13 (Fig. 2A) and T<sub>12</sub>MG and RT-10 (Fig. 2B). Amplified cDNA fragments were resolved by electrophoresis in a 6% denaturing polyacrylamide gel. cDNA fragments that were subsequently isolated (DDRT15, DDRT16 and DDRT25) are indicated by *arrows*.

Figs. 3A and 3B. **Northern blot analysis showing differential expression of selected cadmium-responsive genes.** *C. elegans* poly(A<sup>+</sup>) RNA (2μg), isolated from nematodes exposed to 100μM CdCl<sub>2</sub> for 24 h (+) or control nematodes (-), was resolved by denaturing agarose gel electrophoresis. Northern blots were hybridized with <sup>32</sup>P-labeled cDNA probes prepared from the differential display cDNA fragments VL19 (*upper panel* Fig. 3A) or DDRT16 (*upper panel* Fig. 3B). Following PhosphorImager analysis, the probes were removed and the membrane reprobed with a <sup>32</sup>P-labeled DNA fragment homologous to myosin light-chain mRNAs (*lower panels* Figs. 3A and 3B).

Figs. 4A-4C. **Representative reverse-Northern dot blot of differentially expressed genes.** Cloned DNA fragments were amplified and ~100ng of the amplified product was immobilized on triplicate membranes. The membranes were then hybridized with <sup>32</sup>P-labeled cDNAs synthesized from poly(A<sup>+</sup>) RNA prepared from either untreated *C. elegans* (Fig. 4A), or those exposed to cadmium for 8h (Fig. 4B) or 24h (Fig. 4C). The location of each differentially expressed DNA fragment, and the myosin light-chain (MLC) and metallothionein (MTL-1) controls, on the blots is presented in the following grid legend:



| R<br>o<br>w | A | Column |         |         |        |         |         |        |
|-------------|---|--------|---------|---------|--------|---------|---------|--------|
|             |   | 1      | 2       | 3       | 4      | 5       | 6       | 7      |
|             |   | MLC    | DDRT1   | DDRT2   | DDRT3  | DDRT4   | DDRT5   | DDRT6  |
|             |   |        |         |         |        |         |         | DDRT7  |
| B           |   | DDRT9  | DDRT10  | DDRT12  | DDRT15 | DDRT16  | DDRT17  | DDRT18 |
| C           |   | DDRT20 | DDRT21U | DDRT21D | DDRT22 | DDRT23  | DDRT24  | DDRT25 |
| D           |   | DDRT28 | DDRT29  | DDRT30  | DDRT32 | DDRT33U | DDRT33D | DDRT34 |
| E           |   | DDRT36 | DDRT37  | DDRT38  | DDRT40 | DDRT41  | DDRT47  | DDRT48 |
| F           |   | VL3    | VL9     | VL11    | VL19   | VL20    | VL21    | MTL-1  |

Fig. 5. Sequences corresponding to GenBank Accession Nos. shown in

Table III.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention results, at least in part, from the realization that the non-parasitic nematode *Caenorhabditis elegans* provides an excellent model system for obtaining an integrated picture of cellular, developmental and molecular aspects of the regulation of metal-responsive gene expression (e.g., transition and heavy metal responsive gene expression, including cadmium, mercury, copper, zinc, nickel, lead, chromium, and silver responsive gene expression). The adult hermaphrodite is composed of 959 somatic cells, but contains highly differentiated muscle, nervous, digestive and reproductive systems (Sulstion, J. (1988) in *The Nematode Caenorhabditis elegans* (Wood, W. B., ed) pp. 123-155, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.; Kenyon, C. (1988) *Science* 240, 1448-1453). The developmental and cellular biology of *C.*

*C. elegans* is thoroughly understood in exceptional detail (Sulstion, J. (1988) in *The Nematode Caenorhabditis elegans* (Wood, W. B., ed) pp. 123-155, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.; Kenyon, C. (1988) *Science* 240, 1448-1453). High levels of evolutionary conservation between *C. elegans* and higher organisms are observed in many signal transduction, gene regulatory and developmental pathways (McGhee, J.D. et al. (1997) in *C. elegans II* (Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R., eds) pp. 147-184, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.; Han, M. et al. (1990) *Cell* 65, 921-931; Clark, S.G. et al. (1992) *Nature* 356, 340-344). In addition, homologues of many of the proteins induced as part of metal-activated stress-responses in vertebrates have been identified in *C. elegans*. These include metallothionein (Slice, L.W. et al. (1990) *J. Biol. Chem.* 265, 256-263; Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564), superoxide dismutase (Giglio, A.M. et al. (1994) *Biochem. Mol. Biol. Int.* 33, 41-44; Giglio, M.P. et al. (1994) *Biochem. Mol. Biol. Int.* 33, 37-40), ubiquitin (Zhen, M. et al. (1993) *Mol. Cell. Biol.* 13, 1371-1377; Stringham, E.G. et al. (1992) *Gene* 113, 165-173), heat shock protein 70 (Heschl, M.F.P. et al. (1989) *DNA* 8, 233-243), glutathione-S-transferase (Weston, K. et al. (1989) *Nucleic Acids Res.* 17, 2138-2139) and catalase (Ebert, R.H. et al. (1996) *Dev. Genet.* 18, 131-143). With the exception of metallothionein, the effect of cadmium on the transcription of these *C. elegans* genes remains unknown.

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*C. elegans* also contains homologues to many of the signal transduction proteins that have been implicated in modulating the cellular/molecular response to metal exposure (Gross R.E. et al. (1990) *J. Biol. Chem.* 265, 6896-6907; Lu, X-Y. et al. (1990) *J. Biol. Chem.* 265, 3293-3303; Land, M. et al. (1994) *J. Biol. Chem.* 269, 14820-14827; Land, M. et al. (1994) *J. Biol. Chem.* 269, 9234-9244).

One of the major advantages in using *C. elegans* as a model system, for example, to identify new metal-responsive genes, is the magnitude of cDNA and genomic DNA sequence data currently available. The nematode genome is relatively small ( $\sim 10^8$ bp), and an abundance of information is available on the genetic and physical maps of its chromosomes (Waterston, R.H. et al. in *C. elegans II* (Riddle, D.L., Blumenthal, T., Meyer, B.J. and Priess, J.R., eds) pp. 23-46, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Currently, sequencing of the entire *C. elegans* genome is >80% completed and >50,000 ESTs have been cloned and sequenced. Megabases of genomic and cDNA sequence data are readily available through GenBank, the *C. elegans* Genome Project (Coulson, A. (1996) *Biochem. Soc. Trans.* 24, 289-291) and the *C. elegans* cDNA Sequencing Project (Sequence data and information about the *C. elegans* DNA Project can be obtained at [http://www.ddbj.nig.ac.jp/c-elegans/html/CE\\_INDEX.html/](http://www.ddbj.nig.ac.jp/c-elegans/html/CE_INDEX.html/)).

As described in the Examples that follow, fifty-three differentially expressed DNA fragments from a mixed-stage population (i.e., a population at all stages

of development) of cadmium-exposed *C. elegans* have been identified. Subsequent analysis confirms that the steady-state level of expression of forty-eight of these clones increases 2-6-fold following cadmium exposure. In addition, a single clone was isolated the level of expression of which decreased ~2-fold. Sequence analysis has identified *C. elegans* cosmids, predicted structural genes and ESTs that are identical to the differentially expressed mRNAs. Furthermore, the cadmium-responsive cDNAs are the products of thirty-two independent genes.

With the information provided in the Examples, three types of products, each of which is within the scope of the invention, can be directly obtained:

1. *C. elegans* genes the transcription of which is modulated by cadmium,
2. *C. elegans* mRNAs that are encoded by such genes, and
3. *C. elegans* proteins the expression of which may be affected by cadmium, and subsequently antibodies to these proteins (monoclonal or polyclonal, and antigen binding fragments thereof). These proteins can be expected to function in cadmium detoxification and/or the repair of intra- and intercellular damage.

Based on the BLAST sequence analysis provided in the Examples, the *C. elegans* cadmium-responsive genes can be divided into three categories:

1. *C. elegans* genes that encode proteins that have been shown to be responsive to cadmium in mammals (e.g., metallothionein, pyruvate carboxylase and heat-shock

protein-70);

2. *C. elegans* genes for which mammalian homologues have been identified, but the mammalian genes, etc. have not been shown to be affected by cadmium (e.g., DNA gyrase collagen, human hypothetical protein KIAA0174 [this protein is evolutionarily conserved, it is found in rats and mice] and  $\beta$ -adrenergic receptor kinase); and

3. *C. elegans* genes that encode novel proteins (these predicted proteins do not have any significant homology to any protein currently in the database (e.g., DDRT16)).

Human homologues of proteins in the first two categories can be obtained easily. Using the mRNAs, gene fragments and antibodies derived from the *C. elegans* cadmium-responsive genes in the third category, homologues in higher organisms (e.g., mammals, including rats, mice and humans) of the mRNAs, genes and proteins can be obtained.

While specific reference is made in the Examples that follow to cadmium-toxicity, cadmium-response, etc., for purposes of the present invention, cadmium is functioning as an archetypical stressor. The effects seen with cadmium can be expected to occur with other transition and heavy metals (see above). In addition, other classes of chemical toxins (e.g., chemical carcinogens, oxidizing agents, polycyclic aromatic hydrocarbons) and physical stresses (e.g., ultra-violet light, ionizing radiation, heat-shock, osmotic stress, and infectious agents), can affect the expression of these genes. Accordingly, it will be appreciated that

the embodiments of the invention described below encompass stressors in addition cadmium.

### Biomonitors

The invention includes within its scope biomonitor kits that can contain primers that can be used to amplify specific cadmium-responsive mRNAs in PCRs, or sequence-specific oligonucleotides for Northern blot and Rnase protection assays. Such kits can also contain antibodies specific for the responsive proteins. The kits can be used to assay levels of protein using, for example, Western blot or ELISA assays.

In accordance with this embodiment, the presence of cadmium responsive mRNAs can be determined and the levels of expression of the cadmium-responsive mRNAs or proteins measured in nematodes, indigenous species or humans in potentially contaminated environments to determine if exposure to cadmium has occurred.

In addition, the effectiveness of different therapies used to treat exposure to metals or other chemical toxins can be monitored (i.e., a diagnostic tool for measuring toxicity or stress). If the expression of these genes is found to be associated with disease states, then by monitoring the levels of the protein or mRNA the progression or remission of the disease can be followed. Further, if the expression of the cadmium-responsive genes is modulated by chemotherapeutic agents, as occurs with heat shock proteins and metallothionein, then the affect of these agents on the tumor and the patient can be monitored.

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## Transgenic Organisms (Plants and Animals)

The invention also includes within its scope *C. elegans* or other organisms, the genome of which has been engineered to include a cadmium-responsive gene. The gene can be modified to express a reporter protein (e.g.,  $\beta$ -galactosidase or green fluorescent protein) in place of the normal structural gene. These organisms can be exposed to potentially contaminated environmental samples, water or dirt. The level of reporter gene expression will be proportional to the amount of contamination in the sample. These organisms, which themselves are biomonitors, can be used to measure the levels of bioavailable cadmium and determine the effectiveness of clean-up efforts.

It is expected that certain of the cadmium-responsive genes encode proteins that function in the detoxification and repair of cadmium-induced cellular damage. Over-expression of these proteins would result in the organism being resistant to metal toxicity. Transgenic plants that express the *C. elegans* cadmium-responsive proteins can be generated by controlling the expression the cadmium-responsive mRNA using plant or bacterial promoters. Plants that express these proteins can be expected to be resistant to metal toxicity and thus able to grow in contaminated environments.

There is the potential that disruption of one or more of the cadmium-responsive genes in mammals (i.e., preventing the expression of the native/functional protein) could mimic a human disease state. For example, disruption of VL19 (pyruvate carboxylase) may

mimic a liver disease. A transgenic organism that functions as a disease model constitutes an important tool in the pharmaceutical and medical industries.

### Drug Discovery

The ability to monitor the levels of expression of the cadmium-responsive proteins can be used in drug discovery. Drugs that modulate the expression of these proteins in humans can be expected to function as modulators of other forms of stress. Chemicals or drugs that can be used to increase the expression of the cadmium-responsive proteins can be expected to protect the organism from other stresses. (For example, a drug that increases the expression of one or more of the cadmium responsive genes may allow a patient to receive a higher dose of a chemotherapeutic drug.) A drug that inhibits the ability of cadmium to induce the expression of the mammalian homologues of the *C. elegans* genes can be expected to function in the prevention of cadmium toxicity or other stress-induced toxicities.

The invention includes within its scope drugs discovered using the methods described herein.

### Pathways

The ability of cadmium to induce the transcription of the *C. elegans* genes is likely to be the result of the metal activating intracellular signaling pathways. These pathways ultimately activate transcription factors, which interact with the cadmium-responsive genes. For example, cadmium is taken up by the cell, it



mimic a liver disease. A transgenic organism that functions as a disease model constitutes an important tool in the pharmaceutical and medical industries.

### Drug Discovery

The ability to monitor the levels of expression of the cadmium-responsive proteins can be used in drug discovery. Drugs that modulate the expression of these proteins in humans can be expected to function as modulators of other forms of stress. Chemicals or drugs that can be used to increase the expression of the cadmium-responsive proteins can be expected to protect the organism from other stresses. (For example, a drug that increases the expression of one or more of the cadmium responsive genes may allow a patient to receive a higher dose of a chemotherapeutic drug.) A drug that inhibits the ability of cadmium to induce the expression of the mammalian homologues of the *C. elegans* genes can be expected to function in the prevention of cadmium toxicity or other stress-induced toxicities.

The invention includes within its scope drugs discovered using the methods described herein.

### Pathways

The ability of cadmium to induce the transcription of the *C. elegans* genes is likely to be the result of the metal activating intracellular signaling pathways. These pathways ultimately activate transcription factors, which interact with the cadmium-responsive genes. For example, cadmium is taken up by the cell, it

then binds to an intracellular receptor, this binding activates a series of protein phosphorylations and dephosphorylations and in the end the cadmium responsive gene is "turned-on." It is known that cadmium is a mammalian carcinogen and teratogen. However, the mechanisms that control these effects have not been discovered. A pathway that regulates the expression of the cadmium-responsive genes may represent new a pathway for the development of tumors or other disease. It may also define new pathways that control cell growth and differentiation.

Cadmium-responsive mRNA and protein probes that can be used in the identification of these pathways are within the scope of the invention. Such mRNA and protein probes can be used to monitor the effectiveness of drugs that interact with components of these pathways.

The non-limiting Examples that follow describe certain aspects of the invention in greater detail.

#### EXAMPLES

The following experimental details are referenced in the specific Examples that follow.

*Growth and isolation of C. elegans* - The N2 strain of *C. elegans* was grown in liquid S medium (0.1M NaCl, 50mM potassium phosphate, pH 6.0, 5µg/ml cholesterol, 10mM potassium citrate, 3mM CaCl<sub>2</sub>, 3mM MgCl<sub>2</sub>, 50µM EDTA, 25µM FeSO<sub>4</sub>, 10µM MnCl<sub>2</sub>, 10µM ZnSO<sub>4</sub> and 1µM CuSO<sub>4</sub>) using *E. coli* OP50 as a food source (Brenner, S. (1974) *Genetics* 77, 71-94). In experiments where nematodes

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were exposed to cadmium, the medium was supplemented with 100 $\mu$ M CdCl<sub>2</sub> (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564). *C. elegans* were grown in the presence of metal for 8h or 24h at ~20°C. Nematodes were then collected following centrifugation at 800xg for 5 min. Pellets were suspended in 50mM NaCl containing 35% sucrose (final concentration) and viable nematodes were collected from the top of the solution following centrifugation at 1000xg for 5 min at 4°C. Nematodes were then washed three times by suspension in M9 buffer (22mM KH<sub>2</sub>PO<sub>4</sub>, 42mM Na<sub>2</sub>HPO<sub>4</sub>, 85mM NaCl, 1mM MgSO<sub>4</sub>) followed by sedimentation at 800xg. Washed nematode pellets were finally suspended in a small volume of M9 buffer, rapidly frozen in liquid nitrogen and stored at -80°C.

*RNA isolation* - Total RNA was isolated from mix-stage populations of *C. elegans* exposed to 100 $\mu$ M CdCl<sub>2</sub> for 8h and 24h and control, non-exposed nematodes. Frozen worms were first ground into a fine powder using a liquid nitrogen-cooled mortar and pestle. Powdered *C. elegans* (200 mg) were then homogenized in 2ml of TRIzol (GIBCO/BRL). RNA was then collected from the aqueous phase following the addition of chloroform, precipitated by adding isopropyl alcohol and then air-dried. The dried RNA pellet was then dissolved in diethyl pyrocarbonate (DEPC)-treated water. For some experiments, poly(A<sup>+</sup>) RNA was subsequently isolated using the Poly(A) Tract System following manufacturer's instructions (Promega).

*mRNA Differential display* - Differential display

was performed following the protocol of Liang and Pardee (Liang, P. et al. (1992) *Science* 257, 967-971).

Briefly, 50µg of total RNA isolated from either of three populations of *C. elegans*, controls or those grown in the presence of cadmium for 8h or 24h, was treated with 10 units of RNase-free DNase I (Boehringer Mannheim) in 10mM Tris-Cl buffer, pH 8.3, containing 50mM KCl and 1.5mM MgCl<sub>2</sub>. The DNA-free RNA was precipitated with ethanol and dissolved in DEPC-treated water. First-strand cDNAs were generated in reverse transcriptase reactions containing 0.2µg DNA-free total RNA, reverse transcriptase buffer (25mM Tris-Cl, pH 8.3, 38mM KCl, 1.5mM MgCl<sub>2</sub>, 5mM dithiothreitol), 5µM of each dNTP and 1µM of one of four 3'-degenerate anchored oligo(dT) primers. The 3'-degenerate anchored oligo(dT) primers have the sequence: T<sub>12</sub>MG, T<sub>12</sub>MA, T<sub>12</sub>MT, or T<sub>12</sub>MC, where M is 3-fold degenerate for G, A, and C. Primers were annealed to the RNA template by incubating the reaction mixture for 5 min at 65°C, then for 10 min at 37°C. First strand cDNA synthesis was achieved following the addition of 100 units of Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL) and incubating at 37°C for 50 min. The reaction was terminated by heating at 95°C for 5 min, which inactivates the reverse transcriptase.

Amplification of cDNA fragments was performed in 20µl reactions. Each PCR mixture contained 2µl of the products from one of the four above reverse transcriptase reactions and 18µl of a solution containing *Taq*-PCR buffer (10mM Tris-Cl, pH 8.4, 50mM KCl, 1.5mM MgCl<sub>2</sub>, 0.01% gelatin), 1µM of the same 3'-

degenerate anchored oligo(dT) primer used in the first-strand synthesis reaction, four dNTPs (2μM each), 10μCi [ $\alpha$ -<sup>35</sup>S]dATP (Amersham), 1 unit AmpliTaq DNA polymerase (Perkin-Elmer) and 0.2μM of one of twenty 5'-arbitrary decamers. The sequences of the 5' arbitrary primer used in these reactions are presented in Table I. Reaction mixtures were subjected to 40 cycles of the PCR using the following parameters: denature at 94°C for 30 seconds, anneal at 42°C for 2 min, elongate at 72°C for 30 seconds. All PCRs were performed in duplicate. The amplified cDNAs produced from duplicate reactions of RNA isolated from control, 8h-treated and 24h-treated *C. elegans* were size fractionated in parallel by polyacrylamide gel electrophoresis in 6% acrylamide/8M urea gels.

Table I  
Sequences of the 5'-Arbitrary Decamer Primers used in  
Differential Display

| Primer<br>Designation | Sequence   |
|-----------------------|------------|
| AP-3                  | AGGTGACCGT |
| AP-4                  | GGTACTCCAC |
| AP-6                  | GCAATCGATC |
| AP-7                  | CCGAAGGAAT |
| AP-8                  | GGATTGTGCG |
| AP-9                  | CGTGGCAATA |
| AP-10                 | TAGCAAGTGC |
| AP-13                 | AGTTAGGCAC |
| AP-15                 | AGGGCCTGTT |
| AP-18                 | CTGAGCTAGG |
| RT-1                  | TACAACGAGG |
| RT-2                  | TGGATTGGTC |
| RT-3                  | CTTTCTACCC |
| RT-4                  | TTTTGGCTCC |
| RT-5                  | GGAACCAATC |
| RT-6                  | AAACTCCGTC |
| RT-7                  | TCGATACAGG |
| RT-8                  | TGGTAAAGGG |
| RT-9                  | TCGGTCATAG |
| RT-10                 | GGTACTAAGC |

Following electrophoresis, gels were dried onto Whatman 3MM paper and exposed to Kodak X-AR film for 24h. Differentially expressed cDNAs were visualized by autoradiography. To isolate differentially expressed cDNA fragments, regions of dried gels corresponding to the cDNAs were excised. Gel slices were rehydrated in 100µl dH<sub>2</sub>O following a 10-min incubation at room

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temperature. The cDNA was then extracted from the rehydrated gels by incubating at 100°C for 15 min in tightly capped microcentrifuge tubes. cDNA was recovered by ethanol precipitation in the presence of 0.3M sodium acetate and 50µg of glycogen (Boehringer Mannheim). The eluted cDNA was reamplified in a 40µl reaction with the identical pair of primers used in the mRNA differential display reaction. PCR reaction conditions were similar to those above except, the concentration of the dNTPs was increased to 20µM and the [ $\alpha$ -<sup>35</sup>S]dATP was omitted. Amplified cDNA fragments were resolved by gel electrophoresis using a 1.5% agarose gel and then purified using QIAEXII kits (QIAGEN).

*Subcloning and DNA sequence analysis* - Gel-purified cDNAs were directly inserted into the T-A cloning vector pGEM-T (Promega). DNA inserts were subsequently sequenced using T7 and SP6 primers by the dideoxynucleotide chain termination procedures of Sanger et. al (Sanger, F. et al. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 5463-5467) (United States Biochemicals Sequenase Kit, Version 2.0).

*Computer analysis* - Analysis of cDNA sequence data including sequence comparisons, alignments and generation of contigs were performed using PC/GENE-Intelli-Genetics software. BLAST analysis (Altschul, S.F. et al. (1990) *J. Mol. Biol.* 215, 403-410) was carried out through the National Center for Biotechnology Information and the *C. elegans* Genome Project Internet servers using the non-redundant, *C. elegans* genome and *C. elegans* EST databases. For some

sequence analysis the "A *C. elegans* database" (ACeDB) software was used (Eeckman, F.H. et al. (1995) *Methods Cell. Biol.* 48, 583-605). Predicted *C. elegans* genes were identified by the *C. elegans* Genome Project using the GENEFINDER program (Favella, A. et al. (1995) *Methods Cell. Biol.* 48, 551-569).

*Northern Blot Analysis*—Samples of total RNA (20µg) or poly(A<sup>+</sup>) RNA (2µg) were denatured in a 2.2M formaldehyde/50% (v/v) formamide buffer and then subjected to denaturing gel electrophoresis on a 1.5% agarose/2.2M formaldehyde gel. Size-fractionated RNAs were then transferred to Nytran membrane (Schleicher and Schuell). Membranes were probed with <sup>32</sup>P-labeled cDNA fragments of the differentially expressed mRNAs. cDNAs to be used as probes were generated by the PCR from the cloned DNA fragments recovered from differential display gels. cDNAs were labeled with [ $\alpha$ -<sup>32</sup>P]dCTP (Amersham) by random-primed labeling. Membranes were hybridized in 6 X SSC (1 X SSC = 0.15M sodium chloride, 15mM sodium citrate, pH 7.0), 1.25x Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 300ng denatured sonicated salmon sperm DNA and heat-denatured probe at 42°C for 16h. Following hybridization, membranes were washed at a high stringency of 50°C for 30 min in 0.1 X SSC/0.1% SDS. The amount of probe hybridizing to the RNA was determined by PhosphorImager analysis (Molecular Dynamic System). After images were obtained, membranes were incubated at 95°C for 1h in 0.1% SDS to remove the bound probe. They were then hybridized with a <sup>32</sup>P-labeled *C. elegans* myosin light-chain probe, which served as a



loading control (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564). As a positive control, membranes were also hybridized to a <sup>32</sup>P-labeled *C. elegans* metallothionein-2 (*mtl-2*) cDNA probe (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564). Quantification of radioactivity was performed using the ImageQuant program (Molecular Dynamic System). Steady-state levels of mRNA expression were all normalized to that of the constitutively expressed myosin light-chain mRNAs (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564; Cummins, C. et al. (1988) *Mol. Cell. Biol* 8, 5334-5349).

*Reverse-Northern dot-blot analysis* - Changes in the steady-state levels of differentially expressed mRNAs in *C. elegans* following cadmium-exposure were also determined by reverse-Northern dot-blot analysis by the modified procedure of Zhang et al. (Cummins, C. et al. (1988) *Mol. Cell. Biol* 8, 5334-5349). Briefly, differentially expressed cDNAs that were previously cloned into pGEM-T were amplified using primers that anneal to the T7 and SP6 RNA polymerase binding sites, which flank the cDNA insert. cDNAs were amplified and subsequently purified using a PCR-spin column (QIAGEN). Approximately 100ng of each amplified cDNA were denatured by mixing with 0.1N NaOH (final concentration) and incubating at 100°C for 5 min. The solution was neutralized following the addition of 3 X SSC (final concentration) and then the volume adjusted to 700 µl with dH<sub>2</sub>O. 200-µl of each sample was applied to one of three Nytran membranes in a Bio-Dot microfiltration

apparatus (BioRad). Membranes were then baked for 30 min at 80°C under vacuum. As positive and loading controls 100ng of *mtl-1* cDNA and myosin light chain DNA were also applied to each membrane, respectively.

Three pools of single-stranded <sup>32</sup>P-labeled cDNA probes were prepared from poly(A<sup>+</sup>) RNA isolated from control, 8h and 24h cadmium-treated nematodes. cDNAs were generated from a mixture of mRNAs in a 25µl reverse transcriptase reactions which contained 2µg poly(A<sup>+</sup>) RNA, 1µg oligo(dT)<sub>18</sub> primer, reverse transcriptase buffer, 800µM dATP, dGTP and dTTP, 4.5µM dCTP, 100µCi [ $\alpha$ -<sup>32</sup>P]dCTP (3000Ci/mmol), 20 units RNase inhibitor and 200 units Moloney murine leukemia virus reverse transcriptase. The reaction mixture was incubated at 37°C for 1h, then at 95°C for 5 min to terminate the reaction. Unincorporated nucleotides were separated from the labeled cDNAs by using a G-25 spin column (Boehringer Mannheim). Equal amounts (5x10<sup>6</sup> cpm/ml) of each radioactive cDNA mixture were heat-denatured and then hybridized separately to one of the three membranes at 42°C for 16h in hybridization buffer. Membranes were washed at a high stringency of 0.1 X SSC, 0.1% SDS at 55°C for 30 min. The amount of <sup>32</sup>P-labeled probe bound to each differentially expressed cDNA was quantified by PhosphorImager analysis and levels of expression of the cognate mRNAs normalized to that of the myosin light-chain mRNA.

#### EXAMPLE 1

##### *Effect of Cadmium on Gene Expression*

The level of the *C. elegans mtl-2* mRNA was measured by Northern blot analysis to confirm that the cadmium-exposure protocol outlined above affects gene expression (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564). A <sup>32</sup>P-labeled oligonucleotide probe that is specific for the 3'-end of the *mtl-2* mRNA was hybridized to a membrane that contained RNA prepared from control *C. elegans* or nematodes exposed to 100μM CdCl<sub>2</sub> for 24h (Fig. 1). The steady-state level of *mtl-2* mRNA increased in response to cadmium exposure to that previously reported (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564). This verified that the cadmium-treatment protocol alters gene expression in *C. elegans* and can be used for the differential display analysis.

#### EXAMPLE 2

##### *Identification of Cadmium-responsive Genes by Differential Display*

mRNA expression patterns of non-treated *C. elegans* and those exposed to cadmium for 8h and 24h were compared by mRNA differential display in order to identify new genes whose transcription is regulated by cadmium. A total of twenty 5'-arbitrary decamers, including five that have sequences that are homologous to the *mtl-1* cDNA, were used. Each of the twenty decamers was paired with one of four 3'-degenerate anchored oligo(dT) primers and used to amplify cDNAs prepared from control and cadmium-treated *C. elegans*. All amplification experiments were performed in duplicate using RNA prepared from independently treated populations of *C. elegans*. This generated a total of 480 separate reactions: three populations of cDNA amplified using eighty combinations of primers, in duplicate.

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To bias against isolating differentially displayed cDNAs that are "false positive" (Liang, P. (1993) *Nucleic Acids Res.* 21, 3269-3275), only cDNAs whose level of expression were affected by cadmium in duplicate experiments were selected for further analysis. In addition, cDNA fragments that have altered levels of expression in both the 8h and 24h cadmium-treated samples were selected. Representative data is presented in Fig. 2. A total of seventy-five differentially expressed cDNA fragments were identified and excised from the gels. Of the cDNAs that were selected for further evaluation, the majority showed an increase in band intensity as a result of cadmium treatment, compared to identical sized DNA fragments from the control sample. A single product, VL9, was identified that showed decreased band intensity in the cadmium-treated *C. elegans* cDNA compared to the control.

Fifty-three cDNAs were successfully extracted from the acrylamide gels, reamplified and cloned. Because of the large number of cDNAs successfully isolated, no further attempt was made to clone the remaining fragments. The cloned cDNA fragments ranged in size 141bp to 326bp (Table II). These cDNAs were subsequently sequenced, and changes in the *in vivo* steady-state level of expression of the cognate mRNAs following cadmium treatment evaluated.

Table II

*Changes in the steady-state level of differentially expressed mRNAs*

| Clone name          | Primers used<br>in PCR   | Size of PCR<br>product (bp) | Fold-change in mRNA levels<br>following cadmium exposure <sup>a</sup> |                        |
|---------------------|--------------------------|-----------------------------|---|------------------------|
|                     |                          |                             | 8 hours   | 24 hours               |
| DDRT1               | T <sub>12</sub> MG/RT-4  | 255                         | 2.2   | 3.2                    |
| DDRT2● <sup>b</sup> | T <sub>12</sub> MG/RT-4  | 214                         | 3.1   | 3.9                    |
| DDRT3▲              | T <sub>12</sub> MC/RT-5  | 162                         | 2.0   | 2.4                    |
| DDRT4▲              | T <sub>12</sub> MC/RT-5  | 187                         | 2.5   | 2.8                    |
| DDRT5               | T <sub>12</sub> MA/RT-5  | 254                         | 3.2   | 3.9                    |
| DDRT6               | T <sub>12</sub> MC/RT-5  | 219                         | 4.7   | 5.0                    |
| DDRT7●              | T <sub>12</sub> MG/RT-6  | 217                         | 3.6   | 4.0                    |
| DDRT9               | T <sub>12</sub> MA/RT-6  | 240                         | 3.5   | 2.8                    |
| DDRT10              | T <sub>12</sub> MC/RT-7  | 212                         | 2.7   | 2.5                    |
| DDRT12              | T <sub>12</sub> MA/RT-7  | 226                         | 3.0   | 3.2                    |
| DDRT15              | T <sub>12</sub> MG/RT-10 | 248                         | 2.8   | 3.1                    |
| DDRT16●             | T <sub>12</sub> MG/RT-10 | 240                         | 4.3   | 4.9 (3.5) <sup>c</sup> |
| DDRT17              | T <sub>12</sub> MA/RT-10 | 200                         | 3.7   | 5.7                    |
| DDRT18              | T <sub>12</sub> MC/RT-10 | 228                         | 2.2   | 2.9                    |
| DDRT19■             | T <sub>12</sub> MT/AP-3  | 322                         | 2.7   | 3.9                    |
| DDRT20■             | T <sub>12</sub> MT/AP-3  | 213                         | 2.6   | 2.8                    |
| DDRT21U             | T <sub>12</sub> MC/AP-4  | 284                         | 2.2   | 2.6                    |
| DDRT21D             | T <sub>12</sub> MC/AP-4  | 188                         | 2.2   | 4.2                    |
| DDRT22              | T <sub>12</sub> MC/AP-4  | 292                         | 2.5   | 3.3                    |
| DDRT23▼             | T <sub>12</sub> MG/AP-4  | 277                         | 3.3   | 3.0                    |
| DDRT24              | T <sub>12</sub> MG/AP-4  | 272                         | 1.3   | 1.5                    |
| DDRT25◆             | T <sub>12</sub> MA/AP-13 | 228                         | 3.9   | 4.4                    |
| DDRT26●             | T <sub>12</sub> MG/AP-13 | 238                         | 4.4   | 4.7                    |
| DDRT28◆             | T <sub>12</sub> MA/AP-13 | 308                         | 1.7   | 1.8                    |
| DDRT29              | T <sub>12</sub> MG/AP-15 | 141                         | 3.9   | 4.4                    |
| DDRT30              | T <sub>12</sub> MA/AP-15 | 289                         | 2.7   | 3.1                    |
| DDRT32▼             | T <sub>12</sub> MT/AP-15 | 252                         | 2.6   | 2.4                    |
| DDRT33U             | T <sub>12</sub> MG/AP-18 | 208                         | 3.0   | 3.5                    |
| DDRT33D             | T <sub>12</sub> MG/AP-18 | 171                         | 3.1   | 3.5                    |
| DDRT34              | T <sub>12</sub> MA/AP-18 | 189                         | 2.4   | 3.0                    |
| DDRT35              | T <sub>12</sub> MT/AP-18 | 314                         | 1.6   | 1.9                    |
| DDRT36+             | T <sub>12</sub> MC/AP-18 | 292                         | 1.1   | 1.1                    |
| DDRT37              | T <sub>12</sub> MC/AP-4  | 267                         | 1.0   | 1.2                    |
| DDRT38▼             | T <sub>12</sub> MC/AP-4  | 238                         | 1.1   | 1.8                    |
| DDRT40              | T <sub>12</sub> MA/AP-8  | 264                         | 1.9   | 2.3                    |
| DDRT41              | T <sub>12</sub> MA/AP-8  | 154                         | 2.2   | 2.1                    |

|         |                          |     |                 |           |
|---------|--------------------------|-----|-----------------|-----------|
| DDRT47+ | T <sub>12</sub> MC/AP-13 | 165 | 1.5             | 1.4       |
| DDRT48▼ | T <sub>12</sub> MG/AP-13 | 232 | 2.1             | 2.6       |
| DDRT50  | T <sub>12</sub> MT/AP-15 | 254 | 2.2             | 2.2       |
| VL1★    | T <sub>12</sub> MT/AP-6  | 217 | (4.7)           | (5.2)     |
| VL3     | T <sub>12</sub> MT/AP-6  | 199 | 1.4             | 2.4       |
| VL5★    | T <sub>12</sub> MT/AP-7  | 216 | ND <sup>c</sup> | ND        |
| VL7★    | T <sub>12</sub> MT/AP-7  | 217 | ND              | ND        |
| VL8★    | T <sub>12</sub> MC/AP-6  | 214 | ND              | ND        |
| VL9     | T <sub>12</sub> MT/AP-6  | 356 | -1.7            | -2.3      |
| VL10★   | T <sub>12</sub> MT/AP-7  | 196 | ND              | ND        |
| VL11    | T <sub>12</sub> MT/AP-7  | 142 | 3.2             | 6.1       |
| VL12★   | T <sub>12</sub> MG/AP-10 | 216 | ND              | 5.2       |
| VL13★   | T <sub>12</sub> MG/AP-9  | 216 | ND              | ND        |
| VL15★   | T <sub>12</sub> MT/AP-10 | 217 | ND              | ND        |
| VL19    | T <sub>12</sub> MG/AP-9  | 285 | 3.6             | 5.0 (2.3) |
| VL20    | T <sub>12</sub> MT/AP-9  | 148 | 2.8             | 5.0       |
| VL21    | T <sub>12</sub> MC/AP-10 | 326 | 2.2             | 2.5 (2.0) |

<sup>a</sup> Steady-state levels of differential expressed RNAs were determined by reverse-Northern blot analysis. Fold-change in expression is relative to non-exposed *C. elegans*. All values have been normalized to the level of myosin light chain mRNA and are the average of three independent experiments.

<sup>b</sup> Clones labeled with identical symbols (●▼◆▲★+) indicate that the differentially expressed cDNAs are derived from the same gene (see Table III).

<sup>c</sup> Values in *parenthesis* were determined by Northern blot analysis.

<sup>d</sup> ND; Not determined.

### EXAMPLE 3

#### *Northern and Reverse-Northern Blot Analyses with Differentially Displayed cDNA Fragments*

Northern blot analysis was initially used to confirm that the differentially expressed cDNA fragments, VL3, VL9, VL11, VL19, VL20, VL21 and DDRT16, represent mRNAs whose steady-state levels change following cadmium exposure *in vivo*. RNA blots were prepared with either size fractionated total RNA or poly(A<sup>+</sup>) mRNA, which were isolated from control and cadmium exposed *C. elegans*, and probed with <sup>32</sup>P-labeled cDNA fragments. Northern blot analysis confirmed that as a result of cadmium treatment, the levels of expression of VL19 and DDRT16 increase 2.3-fold and 3.5-fold, respectively (Fig. 3). There is also a 2-fold increase

in the level of VL21 mRNA. The mRNAs for VL3, VL9, VL11 and VL20 were not detected by Northern blots containing poly(A<sup>+</sup>) mRNA isolated from either control or cadmium-treated *C. elegans*.

Reverse-Northern dot-blot analysis (Zheng, H. et al. (1996) *Nucleic Acids Res.* 24, 2454-2455) was performed as an alternative to traditional Northern blots. In this analysis, all of the differentially expressed clones as well as positive and loading controls were simultaneously examined. Representative results are shown in Fig. 4, and Table II summarizes the quantitative analysis from three separate experiments.

*C. elegans mtl-1* and myosin light chain DNAs were used as controls in the reverse-Northern dot-blot analysis. The *mtl-1* mRNA is induced ~5-fold after cadmium treatment (Fig. 4), while the level of expression of myosin light-chain mRNA remained constant. These results are consistent with those previously reported (Freedman, J.H. et al. (1993) *J. Biol. Chem.* 268, 2554-2564).

Of the fifty-three cDNA fragment isolated, forty-six of the clones corresponded to *C. elegans* RNAs whose level of expression increased ~2- to 5-fold following an 8-h cadmium-treatment, and up to 6-fold after a 24-h exposure (Table II). Two differentially expressed mRNAs, VL3 and DDRT38, do not show any significant change in expression following an 8h cadmium exposure, compared to control nematodes. After a 24h exposure, however, there is an ~2-fold increase in their levels of expression. The level of expression for VL9 decreased ~2-fold in response to cadmium. These results confirm that the changes in the levels RNA observed by differential display analysis reflect the *in vivo* molecular response of *C. elegans* to cadmium. Four of the clones, DDRT24, DDRT36, DDRT37 and DDRT47, did not significantly change their level of expression after either 8h or 24h cadmium exposure (Table II). These clones are defined as false positives.

## EXAMPLE 4

### *Nucleotide Sequencing and Homology Searching*

The nucleotide sequences of the differentially expressed DNA fragments were compared against the *C. elegans* genomic and EST databases. Only seven of the cDNA fragments, DDRT12, DDRT15, DDRT21D, DDRT29, DDRT41, VL3 and VL21 did not show a >95% sequence identity to regions of the *C. elegans* genome (Table III). Forty-four clones were identical to *C. elegans* cosmid and/or yeast artificial chromosome (YAC) sequences. In addition, thirty-nine clones were identical to *C. elegans* ESTs (26 of 39) or predicted genes (32 of 39).

Table III

*Sequence analysis of cadmium-regulated, differentially expressed cDNAs*

| Clone name          | Sequence Identity/Homology <sup>a</sup> |   | GenBank accession no. <sup>h</sup> |
|---------------------|---|---|------------------------------------|
|                     | Cosmid <sup>b</sup>                     | Gene product <sup>c</sup>                             |                                    |
| DDRT1               | T09B4                                   | T09B4.1, CELK00886                                    | AF071359                           |
| DDRT2● <sup>d</sup> | F35E8                                   | F35E8.11  | AF071362                           |
| DDRT3▲              | F35E12                                  | F35E12.7  | AF071382                           |
| DDRT4▲              | F35E12                                  | F35E12.7  | AF071391                           |
| DDRT5               | C56C10                                  | C56C10.12, CELK05910                                  | AF071396                           |
| DDRT6               | W03C9                                   | W03C9.5, CELK06396                                    | AF071397                           |
| DDRT7●              | F35E8                                   | F35E8.11  | AF071398                           |
| DDRT9               | C35D10                                  | ND <sup>e</sup>                                       | AF071399                           |
| DDRT10              | C49C3                                   | ND  | AF071353                           |
| DDRT12              | ND                                      | ND  | AF071354                           |
| DDRT15              | ND                                      | ND  | AF071355                           |
| DDRT16●             | F35E8                                   | F35E8.11  | AF071356                           |
| DDRT17              | C49A9                                   | C49A9.4, CELK02276                                    | AF071358                           |
| DDRT18              | F13G4                                   | F13G3.4, CELK06645                                    | AF071360                           |
| DDRT19■             | ZK849                                   | ND  | AF071361                           |
| DDRT20■             | ZK849                                   | ND  | AF071363                           |
| DDRT21U             | Y111B2 <sup>f</sup>                     | ND  | AF072438                           |
| DDRT21D             | ND                                      | CELK05123   | AF071364                           |
| DDRT22              | F57G9                                   | ND  | AF071365                           |
| DDRT23▼             | F31C3                                   | <i>C. elegans</i> rDNA tandem repeats                 | AF071376                           |
| DDRT24              | C56C10                                  | C56C10.8; CELK02788; Human transcription factor BTF3g | AF071377                           |
| DDRT25◆             | R119                                    | R119.5; CELK00686                                     | AF071378                           |



|         |        |   |          |
|---------|--------|---|----------|
| JDRT26● | F35E8  | F35E8.11  | AF071379 |
| DDRT28◆ | R119   | R119.5; CELK00686   | AF071380 |
| DDRT29  | ND     | ND  | AF071138 |
| DDRT30  | C27H5  | C27H5.5; CELK02088; <i>C. elegans</i> collagen ( <i>col-36</i> )        | AF071383 |
| DDRT32▼ | F31C3  | <i>C. elegans</i> rDNA tandem repeats                                   | AF071384 |
| DDRT33D | C34F6  | CELK01885; <i>C. elegans</i> cuticle collagen                           | AF071385 |
| DDRT34  | F20C5  | F20C5.1; CELK01295  | AF071386 |
| DDRT35  | R11D1  | R11D1.1; CELK02809; Human hypothetical protein KIAA0174                 | AF071387 |
| DDRT36+ | D2096  | D2096.8; CELK01725; Human nucleosome assembly protein1<br>LIKE-1        | AF071388 |
| DDRT37  | K11H12 | K11H12.2; CELK02043; Rat 60S ribosomal protein                          | AF071389 |
| DDRT38▼ | F31C3  | <i>C. elegans</i> rDNA tandem repeats                                   | AF071390 |
| DDRT40  | W02B3  | W02B3.2; Bovine $\beta$ -adrenergic receptor kinase                     | AF071392 |
| DDRT41  | ND     | <i>Spiroplasma citri</i> DNA gyrase subunit B                           | AF071393 |
| DDRT47+ | D2096  | D2096.8; CELK01725; Human nucleosome assembly protein1<br>LIKE-1        | AF071394 |
| DDRT48▼ | F31C3  | <i>C. elegans</i> rDNA tandem repeats                                   | AF071395 |
| VL1★    | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF073166 |
| VL3     | ND     | ND  | AF071374 |
| VL5★    | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF071375 |
| VL7★    | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF072436 |
| VL8★    | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF072437 |
| VL9     | C50B6  | ND  | AF071375 |
| VL10    | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF073167 |
| VL11    | C06G3  | C60G3.8   | AF073168 |
| VL12★   | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF072434 |
| VL13★   | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF073169 |
| VL15★   | K11G9  | K11G9.5; CELK3309; <i>C. elegans</i> metallothionein-1( <i>mtl-1</i> )  | AF072435 |
| VL19    | D2023  | D2023.2; CELK00011; Human pyruvate carboxylase                          | AF073170 |
| VL20    | B0228  | B0228.1   | AF071371 |
| VL21    | --     | CELK00200; <i>C. elegans</i> mitochondrial hsp70 protein F<br>precursor | AF071372 |

<sup>a</sup> Analyzed with BLASTN using GenBank and *C. elegans*-specific databases. The sequences have >80% nucleotide sequence identity.

<sup>b</sup> *C. elegans* genomic cosmids that have >90% nucleotide sequence identity.

<sup>c</sup> Predicted genes are designated by the cosmid name followed by the structural gene number (e.g., F35E8.11). *C. elegans* ESTs are denoted with the "CELK" designation.

<sup>d</sup> Clones labeled with identical symbols (●■▼◆▲★+) indicate that the differentially expressed cDNAs are derived from the same gene.

<sup>e</sup> ND, Not detected.

<sup>f</sup> Yeast Artificial Chromosome

<sup>g</sup> Homologous proteins are presented that have a >60% amino acid sequence identity, based on BLASTX analysis.

<sup>h</sup> See Fig. 5

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The results of the BLASTN analysis showed that the differentially expressed cDNA fragments were derived from thirty-two independent genes (Table III). Eight cDNA clones, VL1, VL5, VL7, VL8, VL10, VL12, VL13 and VL15, are identical to the *mtl-1* cDNA sequence. This result is not unexpected because five of the 5'-random decamer primers used in the amplification reactions are identical, or have a one-nt mismatch, to regions in the *mtl-1* cDNA. These primers were specifically selected to function as internal controls which amplified the *mtl-1* cDNA, in order to confirm the efficacy of differential display analysis in identifying cadmium-responsive *C. elegans* genes.

Four clones, DDRT2, DDRT7, DDRT16 and DDRT26, are derived from the predicted gene F35E8.11. They were amplified using the same 3'-degenerate oligo(dT) primer, however, four different 5'-primers were used (Table II). In several cases, pairs of cDNA fragments were isolated that are products of the same gene, DDRT3 and DDRT4; DDRT19 and DDRT20; and DDRT25 and DDRT28. The lengths of the cDNA fragments in each pair are different. Each pair of cDNA products was, however, amplified using identical pair of primers (Table II). Four cDNA fragments were isolated that have sequences that are homologous to the *C. elegans* rDNA tandem repeats in cosmid F31C3. The sequences of two of the clones, DDRT23 and DDRT38, are identical. The sequences of these rDNAs are homologous to a region in the cosmid between nt 25265 and 25433. The sequences of clones DDRT48 and DDRT32 are not homologous to the other rDNA clones. They are identical to regions of the cosmid approximately 3kb from the region homologous to DDRT23 and DDRT38, nt 28041-28245 and nt 28634-28887, respectively.

cDNA fragments isolated using the differential display technique typically contain 3'-untranslated regions of the mRNAs. In order to identify proteins that are encoded by these mRNAs, we took advantage of *C. elegans* cDNA Project data.

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The nucleotide sequences of many of the cadmium-responsive mRNAs are identical to cDNA clones isolated and sequenced by the Project. By assembling contigs consisting of the differentially expressed cDNA sequence and the related *C. elegans* EST sequences, longer open-reading frames were generated. For example, the differentially expressed clone DDRT33D is 171bp and its sequence is 100% identical to the 3'-end of the *C. elegans* clone yk58b1. This clone is a member of a group of six related cDNA clones (cDNA group: CELK01885) for which 3' and 5' sequence data is available. This EST data was collected and assembled into a single 880bp contig that was analyzed by BLASTX (the clones within the CELK01885 cDNA group are yk364f10 (GenBank Accession Number C69593 and GenBank Accession Number C58303), yk279e3 (GenBank Accession Number C68110 and GenBank Accession Number C57084), yk363b2 (GenBank Accession Number C69396 and GenBank Accession Number C58214), yk146f5 (GenBank Accession Number C10425), yk92a12 (GenBank Accession Number D66109 and GenBank Accession Number D69777) and yk58b1 (GenBank Accession Number D65495 and GenBank Accession Number D68941)). This protocol was used for the analysis of clones DDRT1, DDRT21D, DDRT24, DDRT25, DDRT35, DDRT36, VL19 and VL21

The results of the BLASTX analysis are presented in Table III. Cadmium exposure causes an increase in the steady-state levels of a several *C. elegans* proteins that are homologous to proteins in the protein databases. Clone VL21 corresponds to a mRNA that encodes the *C. elegans* HSP70F protein precursor. The expression of HSP70 has been shown to increase following cadmium exposure in mammalian cells (Wiegant. F.A. et al. (1994) *Toxicology* 94, 143-159; Hiranuma, K. et al. (1993) *Biochem. Biophys. Res. Commun.* 194, 531-536). This response, however, has not been reported in *C. elegans*. Cadmium exposure also induced the expression of a mRNA that encodes a DNA gyrase homologue, DDRT41. The

metal caused a 3-fold increase in the levels of mRNAs DDRT30 and DDRT33D that encode two different *C. elegans* collagens (*col-36* and a predicted cuticle collagen, respectively). It also affected mRNAs that encode proteins that are homologous to a  $\beta$ -adrenergic receptor kinase (DDRT40), pyruvate carboxylase (VL19) and the hypothetical human protein KIAA0174 (DDRT35). BLASTX analysis of the remaining differentially expressed cDNAs did not find significant homologies between the translated sequences and those in the non-redundant GenBank database. Thus, the majority of the cadmium responsive, differentially expressed cDNAs encode novel proteins.

\* \* \* \* \*

All documents cited above are hereby incorporated in their entirety by reference, as are all sequences referenced by accession number (e.g., GenBank Accession Number).

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

WHAT IS CLAIMED IS:

1. An isolated *C. elegans* gene the transcription of which is modulated by cadmium.
2. An isolated *C. elegans* mRNA encoded by the gene according to claim 1.
3. An isolated *C. elegans* protein the level expression of which is modulated by cadmium.
4. A *C. elegans* the genome of which has been engineered to include a cadmium-responsive gene.

ABSTRACT OF THE DISCLOSURE

The present invention relates, in general, to stressor-responsive genes and, in particular, to metal-responsive genes, to mRNAs, to proteins encoded therein and to uses thereof, for example, as biomonitors and in drug discovery.

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Figure 1

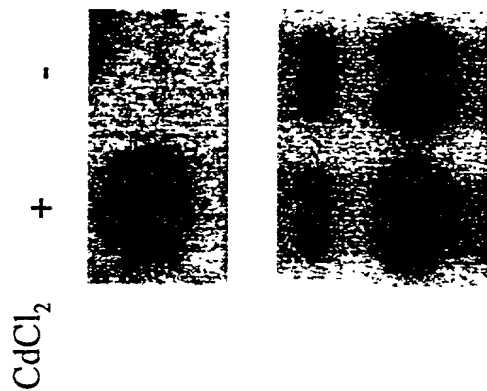
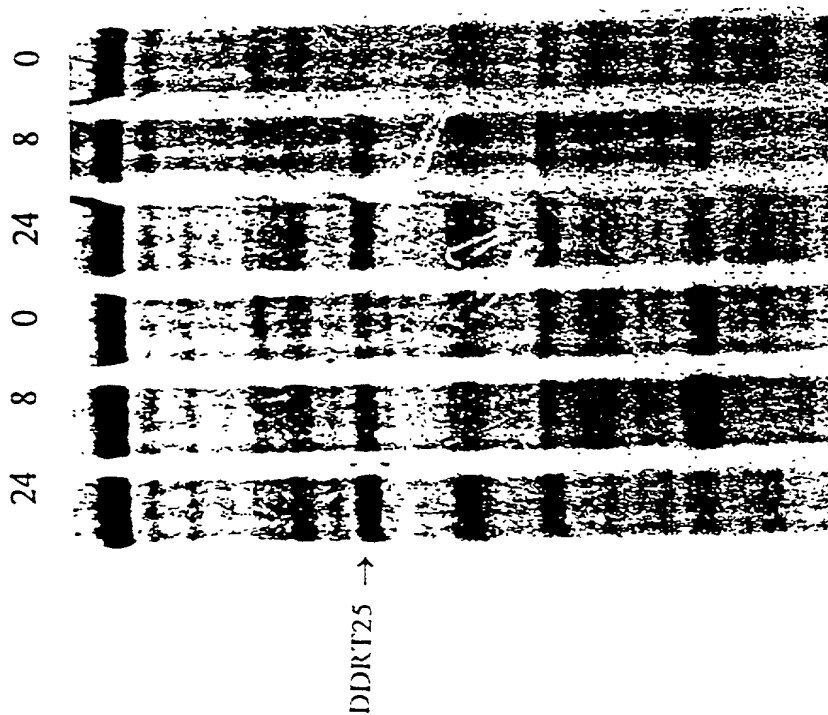
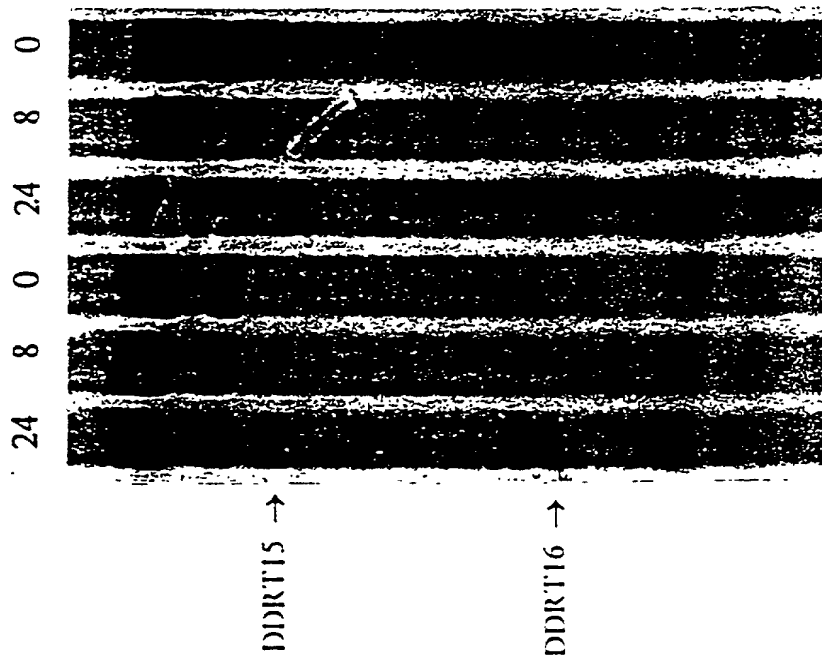


Figure 2  
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A



B



F.12

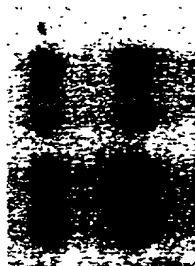


# Figure 3

A

VL19

CdCl<sub>2</sub> + -



DDRT16

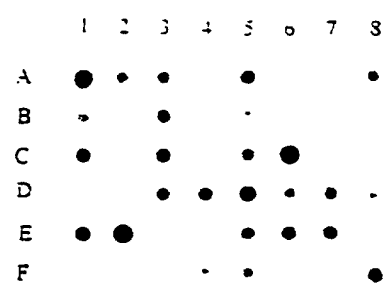
CdCl<sub>2</sub> + -



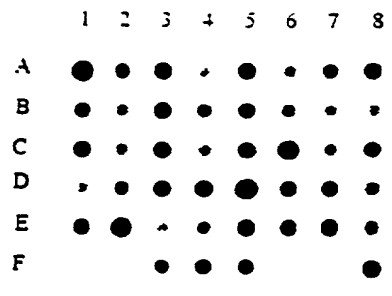
B

F. 4

A



B



C

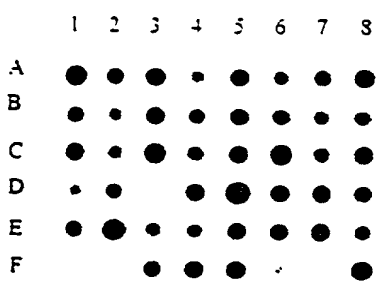


Figure 4

# Figure 5

LOCUS AF072438 284 bp mRNA EST 06-JUL-1998  
DEFINITION AF072438 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT21, mRNA sequence.

ACCESSION AF072438

NID g3288944

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 284)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..284  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT21"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 88 a 61 c 54 g 81 t

ORIGIN

1 ttgtactcca caggacaaa tacattagt ttacaagcc gccacgcgac acgcaacggc  
61 cgtaaatcta cccaaggtag aacaacaaca tgtcaagcac agacccatat cttattgtg  
121 cgggaaggat gcctctactg tagtaatga caattggact cttatccacc ggatcactta  
181 acctattttg atattaatat gcctgattgg ggatcacagg gttgcccgaa aatgtaatt  
241 atgaactgaa ttcgaaatgt attataaat tagttttat tggg

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LOCUS AF072437 214 bp mRNA EST 06-JUL-1998  
DEFINITION AF072437 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone VL8, mRNA sequence.

ACCESSION AF072437

NID g3288943

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 214)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by

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differential display  
JOURNAL Unpublished (1998)  
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FEATURES Location/Qualifiers

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/clone="VL8"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 68 a 37 c 66 g 43 t

ORIGIN

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61 aggaggccag tgagaaaaa tgctgtccag ctggatgtaa gggagactgc aagtgtgcaa  
121 actgtcattg tgcagagcag aagcagtgcg agacaagacc catcaacacc agggaactgc  
181 tgcggctcat taaatgttt cagagttgaa tcta  
//

LOCUS AF072436 217 bp mRNA EST 06-JUL-1998

DEFINITION AF072436 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone VL7, mRNA sequence.

ACCESSION AF072436

NID g3288942

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 217)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

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/tissue\_type="whole animal"  
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 61 gtgaggaggc cagtgagaaa aaatgctgtc cagctggatg taaggagac tgcaagtgtg  
 121 caaactgtca ttgtgcagag cagaagcagt gcgagacaag acccatcaac accagggaac  
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LOCUS AF072435 217 bp mRNA EST 06-JUL-1998  
 DEFINITION AF072435 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL15, mRNA sequence.

ACCESSION AF072435

NID g3288941

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 217)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

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 121 caaactgtca ttgtgcagag cagaagcagt gcgagacaag acccatcaac accagggaac  
 181 tgctgaggct cattaataatg ttccagagtt gaatcta

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LOCUS AF072434 216 bp mRNA EST 06-JUL-1998  
 DEFINITION AF072434 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL12, mRNA sequence.

ACCESSION AF072434

NID g3288940

09437450-111099

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 216)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

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121 aaactgtcat tgtgcagagc agaagcagtg cgagacaaga cccatcaaca ccagggaact  
181 gctgcggctc attaaatgt ttcagagttg aatcta

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LOCUS AF071399 240 bp mRNA EST 01-JUL-1998

DEFINITION AF071399 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT9A, mRNA sequence.

ACCESSION AF071399

NID g3265144

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 240)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
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 121 atataaacgt tgcttaatt tatatatgac gttctctatg aatatagcca aaatgatcga  
 181 tatttttaac ccaaaaatca aacatttttg gtatacgaac ctgccttca cggaggttta

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LOCUS AF071398 217 bp mRNA EST 01-JUL-1998

DEFINITION AF071398 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT7, mRNA sequence.

ACCESSION AF071398

NID g3265143

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 217)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
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BASE COUNT    64 a   38 c   42 g   73 t

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121 attggagtca tgtgaaagag ttcgtcatga agtttaccca aaggcatttc atagtgaatt  
 181 aaattgtcaa actagtagtc agatcaataa aattttc

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LOCUS AF071397 219 bp mRNA EST 01-JUL-1998  
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 cDNA clone DDRT6, mRNA sequence.

ACCESSION AF071397

NID g3265142

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 219)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..219  
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 /dev\_stage="mixed population"

BASE COUNT 75 a 38 c 34 g 72 t

ORIGIN

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 121 aagtataaa ttctccaac aagctactgc atgtccttgc actacaatct tctccgacgg  
 181 attcactct cgatcgcgga tcggattct tcattgttg

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LOCUS AF071396 254 bp mRNA EST 01-JUL-1998  
 DEFINITION AF071396 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT5, mRNA sequence.

ACCESSION AF071396

NID g3265141

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 254)

0943450.1.1109



AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
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FEATURES Location/Qualifiers

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/clone="DDRT5"  
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BASE COUNT 79 a 43 c 50 g 82 t

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121 ttcgaaaaa atgtccataa aaagtgtga atgacttttg ttcatctcga agcatacata  
181 cgatcgaaac ggagaaatcg atagatcgaa tcaggataag tggggatact gtattgtcgg  
241 atgaaaacat agac

//

LOCUS AF071394 165 bp mRNA EST 01-JUL-1998

DEFINITION AF071394 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT47, mRNA sequence.

ACCESSION AF071394

NID g3265139

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 165)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

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Email: jonf@duke.edu.

FEATURES Location/Qualifiers

source 1..165  
/organism="Caenorhabditis elegans"

660777-054450-110950

/strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT47"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"  
 BASE COUNT 38 a 37 c 20 g 70 t  
 ORIGIN  
 1 tttttttt tttccaacc ccttcacata ataggcggaa aaccgattgt tgctgttact  
 61 tgttggtgtg tttatccct gacctatcca tattcccttc ttcccaatct ctaaagatat  
 121 acctgaaaac gagtttttg aatacttgat acatttgctc tcac  
 //  
 LOCUS AF071393 154 bp mRNA EST 01-JUL-1998  
 DEFINITION AF071393 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT41, mRNA sequence.  
 ACCESSION AF071393  
 NID g3265138  
 KEYWORDS EST.  
 SOURCE *Caenorhabditis elegans*.  
 ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.  
 REFERENCE 1 (bases 1 to 154)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)  
 COMMENT  
 Contact: Jonathan H. Freedman  
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 FEATURES Location/Qualifiers  
 source 1..154  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT41"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"  
 BASE COUNT 49 a 27 c 40 g 38 t  
 ORIGIN  
 1 tggattgtgc ggggtgtact gccaaagtctg gtcgtgatag aaaacatcag gcgatcatgc  
 61 ctttacgtgg taagatcctg aacgtcgaaa agcaatggaa cataagatct acgaaaatga  
 121 ggagatcaaa aacatgttta cagctttggt ccta  
 //  
 LOCUS AF071392 264 bp mRNA EST 01-JUL-1998  
 DEFINITION AF071392 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT40, mRNA sequence.

0043450 11099

ACCESSION AF071392

NID g3265137

KEYWORDS EST.

SOURCE Caenorhabditis elegans.

ORGANISM Caenorhabditis elegans

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;

Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; Caenorhabditis.

REFERENCE 1 (bases 1 to 264)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode Caenorhabditis elegans.

Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..264

/organism="Caenorhabditis elegans"

/strain="N2"

/db\_xref="taxon:6239"

/clone="DDRT40"

/clone\_lib="mRNA from cadmium-responsive gene"

/tissue\_type="whole animal"

/dev\_stage="mixed population"

BASE COUNT 90 a 47 c 59 g 68 t

ORIGIN

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1  tggattgtgc ggagtataag caaaaatttc tggaaaagtc gggatgatg aagtttgata
61  agatcttcaa tcaaaagctc ggtttctgt tgttaaaga ttccgcagga aaatgtctcc
121 gagagtcctg gtcctcaaat taaattctac gaggcgatca agaatacga gaaaatggag
181 acaccagatg agcgattaac aaaagcacga gaaattatc gatcatcata tacggttgaa
241 ttccgtcgcg caatcgtcac actc
```

//

LOCUS AF071391 187 bp mRNA EST 01-JUL-1998

DEFINITION AF071391 mRNA from cadmium-responsive gene Caenorhabditis elegans  
cDNA clone DDRT4, mRNA sequence.

ACCESSION AF071391

NID g3265136

KEYWORDS EST.

SOURCE Caenorhabditis elegans.

ORGANISM Caenorhabditis elegans

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;

Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; Caenorhabditis.

REFERENCE 1 (bases 1 to 187)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode Caenorhabditis elegans.

Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

0047450.111050

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FEATURES                      Location/Qualifiers  
 source                      1..187  
                               /organism="Caenorhabditis elegans"  
                               /strain="N2"  
                               /db\_xref="taxon:6239"  
                               /clone="DDRT4"  
                               /clone\_lib="mRNA from cadmium-responsive gene"  
                               /tissue\_type="whole animal"  
                               /dev\_stage="mixed population"

BASE COUNT    57 a   22 c   41 g   67 t

ORIGIN

1 aaatttttat taaaataaaa taaacatgtt ttgttgata ttatagcgtt aaagctgaaa  
 61 tgacaatgat tagaaaacca gcagagaata gagatgatgt tccttcggt gtgtttcca  
 121 gtgaacactt gttgcggtgg agcccgtatt tagcgagtgg tagttttga tgtgattggt  
 181 tccaatc

//

LOCUS    AF071389   267 bp   mRNA            EST    01-JUL-1998

DEFINITION   AF071389 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
                  cDNA clone DDRT37, mRNA sequence.

ACCESSION   AF071389

NID           g3265134

KEYWORDS    EST.

SOURCE      *Caenorhabditis elegans*.

ORGANISM    *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE   1 (bases 1 to 267)

AUTHORS    Freedman,J.H. and Liao,H.-C.

TITLE       Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
               Identification and cloning of new cadmium-responsive genes by  
               differential display

JOURNAL    Unpublished (1998)

COMMENT

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FEATURES                      Location/Qualifiers  
 source                      1..267  
                               /organism="Caenorhabditis elegans"  
                               /strain="N2"  
                               /db\_xref="taxon:6239"  
                               /clone="DDRT37"  
                               /clone\_lib="mRNA from cadmium-responsive gene"  
                               /tissue\_type="whole animal"  
                               /dev\_stage="mixed population"

BASE COUNT    66 a   75 c   54 g   72 t

09437450 "11099

ORIGIN

1 tggctactcca cctacaagtt ctacaagttc tacgagggtg tctctgatcga tccattccac  
61 aaggctatcc gtcgtaaccc agacacccaa tggatcacca agcctagttc acaagcaccg  
121 tgagcaaaga ggactcacct ctgctggacg caagttcgtg gactcggaaa gggattgctt  
181 ttctctgcta cccgcggagg atcccaacac caaagttttt ccaccgcga accgataaat  
241 ctgttattt tattttggtt tgggttt

//

LOCUS AF071388 292 bp mRNA EST 01-JUL-1998

DEFINITION AF071388 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT36, mRNA sequence.

ACCESSION AF071388

NID g3265133

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 292)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..292  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT36"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 73 a 57 c 51 g 111 t

ORIGIN

1 tttttttt ttttccaac ccttcacat aaaggcggaa aaccgattgt tgctgttact  
61 tgttgtgtc gtttatccc tgaggatcc atattccgct tctccaatc tctaaagata  
121 tacctgaaaa cgagttgtcg tcgaaatact tgatacatgt tgttttcatc ctggtgtatg  
181 ttgttcgca aattctcat actagttatg ataggatttg aatgagctgg cagagtgcaa  
241 ctttgaactc gaatttcaat attttcgtga tctctcatta agtgatgaat aa

//

LOCUS AF071387 314 bp mRNA EST 01-JUL-1998

DEFINITION AF071387 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT35, mRNA sequence.

ACCESSION AF071387

NID g3265132

KEYWORDS EST.

09437450 11099

SOURCE *Caenorhabditis elegans*.  
 ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.  
 REFERENCE 1 (bases 1 to 314)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)  
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FEATURES Location/Qualifiers  
 source 1..314  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT35"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 86 a 65 c 56 g 107 t

#### ORIGIN

1 tctgagctag gaggtccagg aggaaacaac ggaggagggtg ctggaaatgg tggattcgac  
 61 gattttgatg atttggtctg ccgtttcgaa gaactgaaaa agattaagta atcatcacc  
 121 gacgttccat tcttattaa ctattgtt ctctccacc caattttt ttacgtgtc  
 181 ttttttga tcataaatga gacccccaaa aactagctgt ttcttagtgc atacgttaaa  
 241 accccttag tcattgatta tcattgtata ctcattatc cgaaaaacct ttgacattc  
 301 atcaactagg tttt

//

LOCUS AF071386 189 bp mRNA EST 01-JUL-1998  
 DEFINITION AF071386 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT34, mRNA sequence.

ACCESSION AF071386

NID g3265131

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 189)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

#### COMMENT

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FEATURES                      Location/Qualifiers  
source                      1..189  
                              /organism="Caenorhabditis elegans"  
                              /strain="N2"  
                              /db\_xref="taxon:6239"  
                              /clone="DDRT34"  
                              /clone\_lib="mRNA from cadmium-responsive gene"  
                              /tissue\_type="whole animal"  
                              /dev\_stage="mixed population"

BASE COUNT    61 a   26 c   48 g   54 t

ORIGIN

1 tctgagctag ggaccgaaat tcacaaatat ccaattgta ctggatggtg gggatgtgga  
61 cgattaatg gggacaagcc actgaagtgt atgttatttc attcgtaaa tatgaagatg  
121 gaggagagtg aatggggatt ttgcttcttt tgcaaaatgg cctccctatg tacctgaaaa  
181 aaaaaaaaa

//

LOCUS    AF071385    171 bp   mRNA            EST    01-JUL-1998  
DEFINITION   AF071385 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
                  cDNA clone DDRT33D, mRNA sequence.

ACCESSION   AF071385

NID           g3265130

KEYWORDS    EST.

SOURCE      *Caenorhabditis elegans*.

ORGANISM    *Caenorhabditis elegans*  
              Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
              Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE    1 (bases 1 to 171)

AUTHORS    Freedman, J.H. and Liao, H.-C.

TITLE       Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
              Identification and cloning of new cadmium-responsive genes by  
              differential display

JOURNAL    Unpublished (1998)

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FEATURES                      Location/Qualifiers  
source                      1..171  
                              /organism="Caenorhabditis elegans"  
                              /strain="N2"  
                              /db\_xref="taxon:6239"  
                              /clone="DDRT33D"  
                              /clone\_lib="mRNA from cadmium-responsive gene"  
                              /tissue\_type="whole animal"  
                              /dev\_stage="mixed population"

BASE COUNT    57 a   44 c   37 g   33 t

ORIGIN

1 tctgagctag gaaaggacgg agaagatgga gagaacggag ctgctggagc cgctggacca

66077-0546460

61 aagggatctt gcgaccactg cccaccacca cgcactcccc aggatattaa ttcacttctc  
121 tctaatttta gtgaatctca ttctaataaa aagccgcccc aaaaaaaaaa a

//

LOCUS AF071383 289 bp mRNA EST 01-JUL-1998  
DEFINITION AF071383 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT30, mRNA sequence.

ACCESSION AF071383

NID g3265128

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 289)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..289  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT30"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 68 a 64 c 63 g 94 t

ORIGIN

1 tttttttt ttgagcgag cgtttattat ttgagtcgag cttgggttga gtcgtcagct  
61 gaacatgaag attgacaaag aagacgatca gcagcaacag atgcgcagag tcgcattctt  
121 tgcgggttct gtctcaactg cagccgtcat ttcaagcatc gtgactctcc caatgatcta  
181 ctcttactct tcaatcttcc caatccatt tgatcattgg aaaccgagtt ctgtaaaact  
241 gtgctcgtga tatgtgtgtg cgaagtctc cacaagtcag gtgtaccct

//

LOCUS AF071382 162 bp mRNA EST 01-JUL-1998  
DEFINITION AF071382 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT3, mRNA sequence.

ACCESSION AF071382

NID g3265127

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

09437450-111099



REFERENCE 1 (bases 1 to 162)  
 AUTHORS Freedman,J.H. and Liao,H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)  
 COMMENT  
 Contact: Jonathan H. Freedman  
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FEATURES Location/Qualifiers  
 source 1..162  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT3"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 64 a 31 c 18 g 49 t

ORIGIN  
 1 ctccaccgca acaagtgttc acgtggaaac aacaacgaaa ggaacatcat ctctatctct  
 61 gctggttttc taatcattgt catttcagct ttaacgctat aatcaacaaa aacagtttat  
 121 tttatttaa taataattta ttcgtgcaaa aaaaaaaaaa aa  
 //

LOCUS AF071381 140 bp mRNA EST 01-JUL-1998  
 DEFINITION AF071381 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT29, mRNA sequence.  
 ACCESSION AF071381  
 NID g3265126  
 KEYWORDS EST.  
 SOURCE *Caenorhabditis elegans*.  
 ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 140)  
 AUTHORS Freedman,J.H. and Liao,H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)  
 COMMENT  
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FEATURES Location/Qualifiers  
 source 1..140  
 /organism="Caenorhabditis elegans"  
 /strain="N2"

09437450.1.1099

/db\_xref="taxon:6239"  
 /clone="DDRT29"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 52 a 25 c 29 g 34 t  
 ORIGIN

1 tagggcctgg ttgtgacaat gtgcactaaa atggggcatg aatcacca gcagagttca  
 61 ctaccctaaa gtgtacttat taagagtcaa ctgtgaagta tatgagacat ttcagttgcc  
 121 tgcccaaaaa aaaaaaaaaa

//

LOCUS AF071380 308 bp mRNA EST 01-JUL-1998  
 DEFINITION AF071380 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT28, mRNA sequence.

ACCESSION AF071380

NID g3265125

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 308)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

COMMENT

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 Email: jonf@duke.edu.

FEATURES Location/Qualifiers

source 1..308  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT28"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 88 a 70 c 73 g 77 t

ORIGIN

1 tagtaggca caggatgtac gaggaaatc tactatttc gggctcacc acgaaatcac  
 61 aataaccgg atttttagt ggtcccgca cgtgacctg ctggcgctg aggcactcg  
 121 ccgcgacatt cgcgacacg cctacaatc acgtgtcaat cgtcagatt gcgcatcaat  
 181 aatggtgatg aaagtgga atactatat ggatcatgtt caaaggcatc aagctgaaca  
 241 attcgaagag ttgaatcggc gtcgacact ttgatccaag accgtaagaa atttgaagc  
 301 tattggtg

//

LOCUS AF071379 238 bp mRNA EST 01-JUL-1998

09437450.1.1099

DEFINITION AF071379 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT26, mRNA sequence.

ACCESSION AF071379

NID g3265124

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 238)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..238  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT26"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 74 a 43 c 44 g 77 t

ORIGIN

1 tttttttt tttggggag gaaatcacgg ctctggatgc aacagtcttc tctcaattgg  
61 caactgtcta ttatccattc cgcaatcaca ttctggatgt tctcgaaaag gacttcccaa  
121 agttattgga gtactgtgaa agagttcgtc atgaagtta cccaaggac ttactatgt  
181 gaattaaatt gtcaactag tagtcagatc aataaaatt tccgcgcgaa aaaaaaaa

//

LOCUS AF071378 324 bp mRNA EST 01-JUL-1998

DEFINITION AF071378 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT25A, mRNA sequence.

ACCESSION AF071378

NID g3265123

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 324)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

0943450 11099

## COMMENT

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 Email: jonf@duke.edu.

## FEATURES Location/Qualifiers

source 1..324  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT25A"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 102 a 70 c 73 g 79 t

## ORIGIN

1 tagttaggca caggatgtac gaggaattc tactatttc gggctcacc acgaaatcac  
 61 aataacccgg atttttagt ggtccccga cgttgaccta ctggcgcggt caggcactcc  
 121 gccgcgacat tcgccgacac gcctacaatc cacgtgtcaa tcgtcagatt tcgggatcaa  
 181 taatggatg gaaagggtga aatacgtata tggatcatgt tcaaaggcat caagctgaac  
 241 aattcgaaga gtggaatcgg cgtcgacaac ttttgatcc aagaccgtaa gaaattgaa  
 301 agctatttgt gaaaaaaaaa aaaa

//

LOCUS AF071377 272 bp mRNA EST 01-JUL-1998

DEFINITION AF071377 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT24, mRNA sequence.

ACCESSION AF071377

NID g3265122

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 272)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

## COMMENT

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## FEATURES Location/Qualifiers

source 1..272  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT24"  
 /clone\_lib="mRNA from cadmium-responsive gene"

09437450.11099

/tissue\_type="whole animal"  
 /dev\_stage="mixed population"  
 BASE COUNT 91 a 74 c 51 g 56 t  
 ORIGIN  
 1 tgg tactcca cgcagaaaga agaaggtcat ccacaacacc gctactaccg atgacaagaa  
 61 gcttcaaagc aattgaaga aactctctgt caccaacatt ccaggaatcg aggagggtcaa  
 121 catgattaag acgatggaac cggtatccac ttcaacaacc caaaagtcta aacctctgtt  
 181 cccagccaat acctctctgt tcacaggatc agccgataac aagtcagatc actgaaatgt  
 241 ctcccaggga atgctgaact ggtcagagtc ct  
 //

LOCUS AF071395 218 bp rRNA INV 30-JUN-1998  
 DEFINITION *Caenorhabditis elegans* strain N2 clone DDRT48, rRNA sequence.  
 ACCESSION AF071395  
 NID g3265140  
 KEYWORDS  
 SOURCE *Caenorhabditis elegans*.  
 ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.  
 REFERENCE 1 (bases 1 to 218)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 218)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Direct Submission  
 JOURNAL Submitted (09-JUN-1998) Nicholas School of the Environment, Duke  
 University, Box 90328, Durham, NC 27708-0328, USA  
 FEATURES Location/Qualifiers  
 source 1..218  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"  
 /note="from cadmium-responsive gene"  
 /clone="DDRT48"  
 misc\_RNA 1..218  
 /note="similar to *Caenorhabditis elegans* rDNA tandem  
 repeats"  
 BASE COUNT 58 a 58 c 38 g 64 t  
 ORIGIN  
 1 tttttttt ttcgacaag cggggactaa aagcaagctt tcatccacc gatgatacaa  
 61 ggcgttttta gtaccttagg atcgactgac ccacatccaa ctactgtcc acgtggaacc  
 121 cttctccact tcagtcttca aggatcgaac ttgaatatt gctactacca tacgatctgc  
 181 actgacggaa agtccagccg agcctacctc atagttaa  
 //

LOCUS AF071390 238 bp rRNA INV 30-JUN-1998  
 DEFINITION *Caenorhabditis elegans* strain N2 clone DDRT38, rRNA sequence.  
 ACCESSION AF071390

09437450.111066

NID g3265135

KEYWORDS .

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 238)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 238)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Direct Submission

JOURNAL Submitted (09-JUN-1998) Nicholas School of the Environment, Duke  
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FEATURES Location/Qualifiers

source 1..238

/organism="Caenorhabditis elegans"

/strain="N2"

/db\_xref="taxon:6239"

/tissue\_type="whole animal"

/dev\_stage="mixed population"

/note="from cadmium-responsive gene"

/clone="DDRT38"

misc\_RNA 1..238

/note="similar to *Caenorhabditis elegans* rDNA tandem  
repeats"

BASE COUNT 58 a 50 c 58 g 72 t

ORIGIN

1 tttttttt tttgccggg cgggtgtgtac aaccggcagg gacgtaatca acgtgagctg  
61 atgactcgcg ctactaggc attcctcgtt taagggaat aattacaata ccctatcccg  
121 gacatggaag aattcaacg gttaccgat accttcaac acgggaaaac taccgggttg  
181 gacaccatta ggactgacag attgaaagtc ttgtcgatt tgggtgttgg ttgtgcat

//

LOCUS AF071384 253 bp rRNA INV 30-JUN-1998

DEFINITION *Caenorhabditis elegans* strain N2 clone DDRT32, rRNA sequence.

ACCESSION AF071384

NID g3265129

KEYWORDS .

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 253)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 253)

AUTHORS Freedman, J.H. and Liao, H.-C.

66077 0542E460

TITLE Direct Submission  
JOURNAL Submitted (09-JUN-1998) Nicholas School of the Environment, Duke  
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## FEATURES Location/Qualifiers

source 1..253  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"  
/note="from cadmium-responsive gene"  
/clone="DDRT32"  
misc\_RNA 1..253  
/note="similar to Caenorhabditis elegans rDNA tandem  
repeats"

BASE COUNT 45 a 60 c 69 g 79 t

## ORIGIN

1 tagggcctgt tgggtgatgc ttgtccggcg cagttctgtc tgcttgatac ttcgggtga  
61 tggcggacta gtgattgtgc ttcttcggga ccgtttctgg tgtgtgcttg gacctcgggt  
121 ctagtatcct gatcgctcat ctatcaaccg tactgtaacc ggtacgactc agggaaatccg  
181 actgtctaataaaacagag gtgacagatg gtccttgccg acgttaactg tctctgatt  
241 ctccccagtg cac

//

LOCUS AF071376 277 bp rRNA INV 30-JUN-1998  
DEFINITION Caenorhabditis elegans strain N2 clone DDRT23, rRNA sequence.

ACCESSION AF071376

NID g3265121

KEYWORDS .

SOURCE Caenorhabditis elegans.

ORGANISM Caenorhabditis elegans

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; Caenorhabditis.

REFERENCE 1 (bases 1 to 277)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode Caenorhabditis elegans.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 277)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Direct Submission

JOURNAL Submitted (09-JUN-1998) Nicholas School of the Environment, Duke  
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## FEATURES Location/Qualifiers

source 1..277  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"  
/note="from cadmium-responsive gene"  
/clone="DDRT23"  
misc\_RNA 1..277

05437450.11099

/note="similar to Caenorhabditis elegans rDNA tandem repeats"

BASE COUNT 67 a 59 c 73 g 78 t

ORIGIN

1 tttttttt ttggggggg gcggtgtga caaagggcag ggacgtaac aacgtgagct  
61 gatgactcac acttctaggc attcctcgtt taagggaata attacaatac ccatcccgga  
121 catggaagaa ttcaacgggt ttaccgatac ctttcggca acacgggaaa actcaccggg  
181 tccggacacc attaggactg acagattgaa agctctttct cgatttggtg gttggtggtg  
241 catggccgtt cttagtgtg ggagtaccaa tcactag

//

LOCUS AF071375 216 bp mRNA EST 30-JUN-1998

DEFINITION AF071375 mRNA from cadmium-responsive gene Caenorhabditis elegans  
cDNA clone VL5, mRNA sequence.

ACCESSION AF071375

NID g3265120

KEYWORDS EST.

SOURCE Caenorhabditis elegans.

ORGANISM Caenorhabditis elegans

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; Caenorhabditis.

REFERENCE 1 (bases 1 to 216)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode Caenorhabditis elegans.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..216  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="VL5"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 69 a 37 c 66 g 44 t

ORIGIN

1 tagcaagtgc ggagacaaat gtgaatgcag tggagacaag tgtgtgaga agtactgctg  
61 tgaggaggcc agtgagaaaa aatgctgtcc agctggatgt aaggagact gcaagtgtgc  
121 aaactgtcat tgtgcagagc agaagcagtg cgagacaaga cccatcaaca ccagggaact  
181 gctgcggctc attaaaaatg ttcagattg aatcta

//

LOCUS AF071374 356 bp mRNA EST 30-JUN-1998

DEFINITION AF071374 mRNA from cadmium-responsive gene Caenorhabditis elegans  
cDNA clone VL9, mRNA sequence.

ACCESSION AF071374

660777 0542460



NID g3265119

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 356)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..356  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="VL9"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 126 a 70 c 55 g 105 t

ORIGIN

1 caatcgatga gtatcctcgt acaattaatg catgatgcaa ttggaaatat tccgaggtag  
61 gtaaacggg gaacatcacg agatagatga atacagcgga tatcatatag gcacgcagaa  
121 tatcaataaa attttcaaat ttcaaaata tcataacgat tataacacgt agcagggaat  
181 tttaaagcca ctgaaataaa tatagaataa tatatacaga cacacacaat ctagatttca  
241 gaacattttc agtaacgacg ttgaacttt ttgaagatt tcgccgagcc ttgatcact  
301 ttgcagtc caactccac aactttctt tctctctct cctctacatc gattgc  
//

LOCUS AF071373 199 bp mRNA EST 30-JUN-1998

DEFINITION AF071373 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone VL3A, mRNA sequence.

ACCESSION AF071373

NID g3265118

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 199)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

09437450.1.1.099

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FEATURES            Location/Qualifiers  
 source            1..199  
                   /organism="Caenorhabditis elegans"  
                   /strain="N2"  
                   /db\_xref="taxon:6239"  
                   /clone="VL3A"  
                   /clone\_lib="mRNA from cadmium-responsive gene"  
                   /tissue\_type="whole animal"  
                   /dev\_stage="mixed population"

BASE COUNT    55 a   31 c   20 g   93 t

#### ORIGIN

1 taaactctat gtttattgt ttttcaaat ttcaaattga aaattgaaac ttcaatttg  
 61 attagagtct ttgtggttg actcctttt ttattgaac atcttttacg tacgtcatag  
 121 tttgtatac acattacaa atgtgtttt gtaattatat gtaacaaatt tctatgtaca  
 181 cctcatctca tctctctat

//

LOCUS    AF071372   326 bp   mRNA            EST    30-JUN-1998

DEFINITION AF071372 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL21A, mRNA sequence.

ACCESSION AF071372

NID        g3265117

KEYWORDS EST.

SOURCE    *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 326)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

#### COMMENT

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FEATURES            Location/Qualifiers  
 source            1..326  
                   /organism="Caenorhabditis elegans"  
                   /strain="N2"  
                   /db\_xref="taxon:6239"  
                   /clone="VL21A"  
                   /clone\_lib="mRNA from cadmium-responsive gene"  
                   /tissue\_type="whole animal"  
                   /dev\_stage="mixed population"

BASE COUNT    76 a   61 c   59 g   130 t

660777 0542460

## ORIGIN

1 cgtctccctt tttacttac ttgtaggtgc gtcttgtaa ttgtacgtac ttatatttag  
 61 caaacctctg gtgttacctc tgccttttg taaaattgt tacacacttt cttttggca  
 121 gtaaaagttg ttagcacac ttaacactc tgccactacc aaggtaatag tgagcccatc  
 181 gaggttttat aaatgtcctt gatagtttaa agtgttggag gatcgagcta ctttgtagt  
 241 ggaaagccgt gtttctgtc ttgtttgtt cgatgattta cccaactatt tgatatttg  
 301 attaccgga ttatataata caccce

//

LOCUS AF071371 147 bp mRNA EST 30-JUN-1998

DEFINITION AF071371 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL20B, mRNA sequence.

ACCESSION AF071371

NID g3265116

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 147)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

## COMMENT

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FEATURES Location/Qualifiers

source 1..147  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="VL20B"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 54 a 27 c 22 g 44 t

## ORIGIN

1 tggcaatata cctagaaga gtaaatatta tgacgtggca ataatacaga agcagtccga  
 61 actacaactc acgaacatt tgaaagttt acctctgat ttctttgaa tgtttgtct  
 121 cacacaataa agaaaattct accgtac

//

LOCUS AF071370 285 bp mRNA EST 30-JUN-1998

DEFINITION AF071370 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL19, mRNA sequence.

ACCESSION AF071370

NID g3265115

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

05437450.111099

ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.  
 REFERENCE 1 (bases 1 to 285)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)  
 COMMENT

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 Email: jonf@duke.edu.

FEATURES Location/Qualifiers  
 source 1..285  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="VL19"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 91 a 46 c 52 g 96 t

#### ORIGIN

1 cgtggcaata cacagaatat acacattgag atggttcgaa tggcaaagag aaggtggtgg  
 61 ctaatcattc tatatagcac aacgccaaat ataattcga tgtggcggaa ttgtgatg  
 121 tgaatggaat taacaaaatt ttctaacgt cttcattcgg agtaattttt cgtttcct  
 181 ccacttttcg atttatattg ttttctaga aaaagtattt attgcacgg gtgctcattg  
 241 tctttgtgta gaataataac tcgttcactt cccaaaaaaa aaaaa  
 //

LOCUS AF071369 216 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071369 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL13, mRNA sequence.

ACCESSION AF071369

NID g3265114

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 216)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)

#### COMMENT

Contact: Jonathan H. Freedman  
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 Box 90328, Durham, NC 27708-0328, USA

660777 0542460

Email: jonf@duke.edu.

# FEATURES Location/Qualifiers

source 1..216  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="VL13"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 69 a 37 c 66 g 44 t

## ORIGIN

1 tagcaagtcg gagacaaatg tgaatgcagt ggagacaagt gttgtgagaa gtactgctgt  
 61 gaggaggcca gtgagaaaaa atgctgtcca gctggatga agggagactg caagtgtgca  
 121 aactgtcatt gtgcagagca gaagcagtgc ggagacaaga cccatcaaca ccagggaact  
 181 gctgcggctc attaaaatgt ttcagagtgt aatcta

//

LOCUS AF071368 142 bp mRNA EST 30-JUN-1998

DEFINITION AF071368 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL11, mRNA sequence.

ACCESSION AF071368

NID g3265113

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 142)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

## COMMENT

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 Email: jonf@duke.edu.

# FEATURES Location/Qualifiers

source 1..142  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="VL11"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 45 a 21 c 23 g 53 t

## ORIGIN

1 gtgctggagt tgtttgtatt tcagaataaa taaaataaaa tatgatttga gtagaatatt  
 61 aaaataaagt ccttcacatt aaattatcaa ttgcttggtc tcgaatatct tccagctggt  
 121 gattgcattc gttcattcct tc

660777 054450 111099

//

LOCUS AF071367 84 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071367 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL10, mRNA sequence.  
 ACCESSION AF071367  
 NID g3265112  
 KEYWORDS EST.  
 SOURCE *Caenorhabditis elegans*.  
 ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.  
 REFERENCE 1 (bases 1 to 84)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display  
 JOURNAL Unpublished (1998)  
 COMMENT  
 Contact: Jonathan H. Freedman  
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FEATURES Location/Qualifiers  
 source 1..84  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="VL10"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 29 a 10 c 27 g 18 t

ORIGIN

1 tagcaagtcg ggagacaaat gtgaatgcag tggagacaag tgtgtgaga agtactgctg  
 61 tgaggattcc agtgagaaaa aatc

//

LOCUS AF071366 217 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071366 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone VL1, mRNA sequence.  
 ACCESSION AF071366  
 NID g3265111  
 KEYWORDS EST.  
 SOURCE *Caenorhabditis elegans*.  
 ORGANISM *Caenorhabditis elegans*  
 Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.  
 REFERENCE 1 (bases 1 to 217)  
 AUTHORS Freedman, J.H. and Liao, H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..217  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="VL1"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 69 a 37 c 67 g 44 t

ORIGIN

1 tagcaagtgc ggagacaaat gtgaatgcag tggagacaag tgtgtgaga agtactgctg  
61 tgaggaggcc agtgagaaaa aatgctgtcc agctggatgt aaggagact gcaagtgtgc  
121 aaactgtcat tgtgcagagc agaagcagtg cggagacaag acccatcaac accagggaac  
181 tgctgcggct cattaaaatg ttccagagtt gaatcta

//

LOCUS AF071365 292 bp mRNA EST 30-JUN-1998

DEFINITION AF071365 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT22, mRNA sequence.

ACCESSION AF071365

NID g3265110

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 292)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..292  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT22"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"

09437450.1.1099

/dev\_stage="mixed population"  
 BASE COUNT 101 a 60 c 50 g 81 t  
 ORIGIN  
 1 tggtagtcca caccgacaaa tacatttagt ttacaagcc gccacgcgac acgcaacgcc  
 61 gtaaattac caaggtaaa caacaacatg tcaagcacag acccatatct tattgtgcg  
 121 gaacgagatg gcctctactg tagtaatcga caattggact ctatccacc ggatcactta  
 181 acctatttg atattaatat tcctattggg atcacagggt ttgcccga aa atgtaattat  
 241 gaactgaatt gaaatgtatt ataaattagt tttattggg aaaaaaaaa aa

//

LOCUS AF071364 188 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071364 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT21D, mRNA sequence.

ACCESSION AF071364

NID g3265109

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;

Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 188)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.

Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..188

/organism="Caenorhabditis elegans"

/strain="N2"

/db\_xref="taxon:6239"

/clone="DDRT21D"

/clone\_lib="mRNA from cadmium-responsive gene"

/tissue\_type="whole animal"

/dev\_stage="mixed population"

BASE COUNT 44 a 31 c 51 g 62 t

ORIGIN

1 tataggttaa gtgatccggt ggataagagt ccaattgtcg attactacag tagaggccat  
 61 ctgcttccgc acaataaga tatgggtctg tgcttgacat gttgtgttg tacctgggt  
 121 agatttacgg cagttgcgtg tcgttgccgg ctgtgaaac taaatgtatt ttccgtgtg  
 181 gagtacca

//

LOCUS AF071363 289 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071363 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT20, mRNA sequence.

ACCESSION AF071363

NID g3265108

09437450-11099



## KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.ORGANISM *Caenorhabditis elegans*Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 289)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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## FEATURES Location/Qualifiers

source 1..289  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT20"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 63 a 56 c 75 g 95 t

## ORIGIN

1 tttttttt ttgtacat tatggcaaat ggaggcactg tctggtccg tggggtcatg  
61 gtgcattgga tcatgtata tcctatcctg gcttctaate ccaatgcgtt tacagtcag  
121 tgggcttgaa cgggcctagc tgagcttgga caaagttcct tgacagtacg ggctgacaag  
181 ctgacagctc agaaattagg cacttggtggg ctacaggtgc tcgtaattat ttgagagtt  
241 ctgggcttcc ggactttac taggctaate taagacaact gggctctaa  
//

LOCUS AF071362 214 bp mRNA EST 30-JUN-1998

DEFINITION AF071362 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT2, mRNA sequence.

ACCESSION AF071362

NID g3265107

## KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.ORGANISM *Caenorhabditis elegans*Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 214)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES                      Location/Qualifiers  
source                      1..214  
                              /organism="Caenorhabditis elegans"  
                              /strain="N2"  
                              /db\_xref="taxon:6239"  
                              /clone="DDRT2"  
                              /clone\_lib="mRNA from cadmium-responsive gene"  
                              /tissue\_type="whole animal"  
                              /dev\_stage="mixed population"

BASE COUNT    77 a   37 c   40 g   60 t

ORIGIN

1 aaatcatggc ggcggatgca acagtcttct caattggcaa ctgtctatat cattccgcaa  
61 cacatttcgg atgttctcga aaaggacttc ccaaagttat tggagtactg tgaaagagt  
121 cgtcatgaag ttcccaaag gactttacta tgtgaattaa atgtcaaac tagtagtcag  
181 atcaataaaa tttacgtgg aaaaaaaaaa aaaa

//

LOCUS    AF071361   322 bp   mRNA            EST    30-JUN-1998  
DEFINITION   AF071361 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
                 cDNA clone DDRT19, mRNA sequence.

ACCESSION   AF071361

NID           g3265106

KEYWORDS    EST.

SOURCE      *Caenorhabditis elegans*.

ORGANISM    *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE   1 (bases 1 to 322)

AUTHORS    Freedman, J.H. and Liao, H.-C.

TITLE       Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
              Identification and cloning of new cadmium-responsive genes by  
              differential display

JOURNAL    Unpublished (1998)

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FEATURES                      Location/Qualifiers  
source                      1..322  
                              /organism="Caenorhabditis elegans"  
                              /strain="N2"  
                              /db\_xref="taxon:6239"  
                              /clone="DDRT19"  
                              /clone\_lib="mRNA from cadmium-responsive gene"  
                              /tissue\_type="whole animal"  
                              /dev\_stage="mixed population"

BASE COUNT    102 a   77 c   70 g   73 t

ORIGIN

1 taggtgaccg tagagaagcc cagatattta aaatctaaag ggaaactgtt tgaccagaag

09437450.11099

61 attagagccc agttgtctta gatagcctag taaaagtccg gaagcccaga actctcaaaa  
 121 taattacgag cactgttagc ccacaagtgc ctaattctg actgtcaagc ttgtcgacct  
 181 gtactgtcaa ggaactttgt caagctcagc taggccgtt caagcccaca tgactgtaaa  
 241 cgattcggga ttagaagcca ggataggata tccatgatcc aatgcacat gaccacgga  
 301 accagatgtg ctcattacat ag

//

LOCUS AF071360 228 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071360 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT18, mRNA sequence.

ACCESSION AF071360

NID g3265105

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 228)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..228  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT18"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 70 a 49 c 20 g 89 t

ORIGIN

1 tttttttt ttccccatt catcacacac tatcatgtt tatattcaga cctattacct  
 61 gtccagaaaa actgagctga aaaaatccc gacgagcagc tccttcacat tcaaatctt  
 121 ccattcattt cccactcaat tcattgttt tgccttgat ttcaaat ttgccttat  
 181 tattttattg ctaattaag aaaactgtta ctttgcaaaa aaaaaaaaa

//

LOCUS AF071359 255 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071359 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT1, mRNA sequence.

ACCESSION AF071359

NID g3265104

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

09437450.111099

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; Caenorhabditis.

REFERENCE 1 (bases 1 to 255)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
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JOURNAL Unpublished (1998)

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FEATURES Location/Qualifiers

source 1..255  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT1"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 113 a 25 c 51 g 66 t

ORIGIN

1 atcattcaag aaagctatta tcagaaaaca taaatgacat agatcaagtg taaatcacat  
61 atatataaag tggataaata tatatagtta aacggataag gaaattaatt aatgaattt  
121 gaaactggca gcgaaggatg aacagggaaa ggcacatgtt aaaataaatg aatgtgtata  
181 atttcgtgaa gagttagtta tgttagtgta tggcagccat gcagaatgag ccattgttcc  
241 gaaaaaaaaa aaaaaa

//

LOCUS AF071358 433 bp mRNA EST 30-JUN-1998

DEFINITION AF071358 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT17, mRNA sequence.

ACCESSION AF071358

NID g3265103

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 433)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

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Email: jonf@duke.edu.

66071358.054/E460

FEATURES            Location/Qualifiers  
 source            1..433  
                   /organism="Caenorhabditis elegans"  
                   /strain="N2"  
                   /db\_xref="taxon:6239"  
                   /clone="DDRT17"  
                   /clone\_lib="mRNA from cadmium-responsive gene"  
                   /tissue\_type="whole animal"  
                   /dev\_stage="mixed population"

BASE COUNT    149 a   63 c   83 g   138 t

ORIGIN

```

1  tggactaag ggccaataac tgagctttg cacggcggca tcaatgataa agagaaacta
61  ttttgacgg ttaaaataac caaattaca cggcgagtc aatcaaaaat tctcatctgg
121 aacagcaaag tacatcgag aattgctgga aggaagcact gatgaaacta aattaactgc
181 tggatgcata ggaaaaacgt caagattgac gtggagtgg agagaaggac tatgtttgga
241 tggttactaa gattttgtaa ctgtgacaa taaggacatc acttttctaa ctaactaaa
301 tctttttta cttctttct tctgaattaa ttgtgttta aggcgaattc tatgtttcag
361 aattattta cgtttgctt tcatgttta attgtaaaag taagcaattt ttccaccgta
421 aaaaaaaaaa aaa
  
```

//

LOCUS    AF071357   933 bp   mRNA        EST    30-JUN-1998  
 DEFINITION AF071357 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
             cDNA clone DDRT16FC, mRNA sequence.

ACCESSION AF071357

NID        g3265102

KEYWORDS EST.

SOURCE    *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*  
           Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
           Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 933)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE      Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
             Identification and cloning of new cadmium-responsive genes by  
             differential display

JOURNAL    Unpublished (1998)

COMMENT

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FEATURES            Location/Qualifiers  
 source            1..933  
                   /organism="Caenorhabditis elegans"  
                   /strain="N2"  
                   /db\_xref="taxon:6239"  
                   /clone="DDRT16FC"  
                   /clone\_lib="mRNA from cadmium-responsive gene"  
                   /tissue\_type="whole animal"  
                   /dev\_stage="mixed population"

BASE COUNT    275 a   190 c   171 g   297 t

ORIGIN

DDRT16FC:11099

1 catgcattt tttttttt ttttttta ctgtctcaag tatgttggat tcatgttga  
61 ttattactgc tgcgctgtt ggagccgctg tcattactt gaaaaattc ttcactgtc  
121 ctacattaa accaaaacct gatattcaca aaaaagacta caaaaaggat gtatgtctatc  
181 tgtatcagat gaagagactc aagaactgtc cgaactgtc cctttctgc atgaaaatcg  
241 agattctttg tagaatctt aagattcctt acgagattat cacatgcacc tctgaacgt  
301 ctcggaatgg attggtccct ttcgttgaac tcaatggaga gcacattgct gattctgac  
361 ttatcgaaat gcgcttgaga tcacatttta aaattccgtc gcttccaact gagctggaaa  
421 ctcaatctgt tgctctaagc aagtttgag atcaccattt gttcttcgta cttatacgat  
481 ttaaaattgc tgcgacgaa ttctacaaaa ccattatcga aataatcgtt ctccaacct  
541 tctgaattt ccttctcatg ccccttttga aggctataat cgggaaaaat gtctacaaca  
601 aatgtcaggg agccattgga gatttgaat tgagtgaact cgacgagatt cttcacagag  
661 atttgcgaat cgtagagaac acctggcca agaaaaagt tctttcggg gaggaatca  
721 cggcggcgga tgcaacagtc ttcttcaat tggcaactgt ctattatcca ttccgcaatc  
781 acattcgga tgttctcga aaggacttcc caaagtatt ggagtactgt gaaagagttc  
841 gtcatgaagt ttaccaaag gactttacta tgtgaattaa attgtcaaac tagtagtcag  
901 atcaataaaa ttctacgttg caaaaaaaaa aaa

//

LOCUS AF071356 238 bp mRNA EST 30-JUN-1998  
DEFINITION AF071356 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
cDNA clone DDRT16, mRNA sequence.

ACCESSION AF071356

NID g3265101

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 238)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
Identification and cloning of new cadmium-responsive genes by  
differential display

JOURNAL Unpublished (1998)

COMMENT

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Email: jonf@duke.edu.

FEATURES Location/Qualifiers

source 1..238  
/organism="Caenorhabditis elegans"  
/strain="N2"  
/db\_xref="taxon:6239"  
/clone="DDRT16"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 77 a 42 c 45 g 74 t

ORIGIN

1 tttttttt ttggaggga aatcacggcg gcggatgcaa cagtcttc tcaattggca  
61 actgtctatt atccattccg caatcacatt tcggatgttc tcgaaaagga ctcccaaag  
121 tattggagat actgtgaaag agttcgtcat gaagtttacc caaggactt tactatgtga

09437450.1.1099

181 attaaattgt caaactagta gtcagatcaa taaaattcta cgtggcaaaa aaaaaaaa

//

LOCUS AF071355 248 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071355 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT15, mRNA sequence.

ACCESSION AF071355

NID g3265100

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;

Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 248)

AUTHORS Freedman, J.H. and Liao, H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.

Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..248

/organism="Caenorhabditis elegans"

/strain="N2"

/db\_xref="taxon:6239"

/clone="DDRT15"

/clone\_lib="mRNA from cadmium-responsive gene"

/tissue\_type="whole animal"

/dev\_stage="mixed population"

BASE COUNT 67 a 46 c 31 g 104 t

ORIGIN

1 tttttttt tticgcatgt tgtaatacta atatttatta attttcttta attttctttg

61 ttaagtttgt atttataggt tgttgagatt tttttgcctg taattttgca actgtgattc

121 atgtatgtac tatatgaacc gaaacccct cccgtcatat acaacagtta gtaaacatt

181 ttaatcccat atttctcatt cccaacactc ttacagggtt tgcatcagca gcagcagtgc

241 aaacacaa

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LOCUS AF071354 226 bp mRNA EST 30-JUN-1998  
 DEFINITION AF071354 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT12, mRNA sequence.

ACCESSION AF071354

NID g3265099

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;

Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 226)

AUTHORS Freedman,J.H. and Liao,H.-C.  
 TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..226  
 /organism="Caenorhabditis elegans"  
 /strain="N2"  
 /db\_xref="taxon:6239"  
 /clone="DDRT12"  
 /clone\_lib="mRNA from cadmium-responsive gene"  
 /tissue\_type="whole animal"  
 /dev\_stage="mixed population"

BASE COUNT 68 a 38 c 35 g 85 t

ORIGIN

1 tttttttt tttgtaaca aagaactgag cactctatgg ttatcaagt ctatatgtat  
 61 ccgtgatgcc tactgtatcg tacatccatc tcgatcgtaa tgcattattg atcatgagtt  
 121 cccaaaggtc ttaatcttga caaagggtgca atagatatat atcctattt ggccactatat  
 181 atatgttcag aattatgact gatcgataca tatgatcaaa gttaca

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LOCUS AF071353 212 bp mRNA EST 30-JUN-1998

DEFINITION AF071353 mRNA from cadmium-responsive gene *Caenorhabditis elegans*  
 cDNA clone DDRT10, mRNA sequence.

ACCESSION AF071353

NID g3265098

KEYWORDS EST.

SOURCE *Caenorhabditis elegans*.

ORGANISM *Caenorhabditis elegans*

Eukaryota; Metazoa; Nematoda; Secernentea; Rhabditia; Rhabditida;  
 Rhabditina; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*.

REFERENCE 1 (bases 1 to 212)

AUTHORS Freedman,J.H. and Liao,H.-C.

TITLE Cadmium-Regulated Genes from the Nematode *Caenorhabditis elegans*.  
 Identification and cloning of new cadmium-responsive genes by  
 differential display

JOURNAL Unpublished (1998)

COMMENT

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FEATURES Location/Qualifiers

source 1..212  
 /organism="Caenorhabditis elegans"  
 /strain="N2"

660777 0542E460



/db\_xref="taxon:6239"  
/clone="DDRT10"  
/clone\_lib="mRNA from cadmium-responsive gene"  
/tissue\_type="whole animal"  
/dev\_stage="mixed population"

BASE COUNT 68 a 35 c 34 g 75 t

ORIGIN

1 ttcgatacag gaactacatt tacatctgtt tcaacatac aacaatacat aacatactca  
61 atccttcagg ctctgaagga tttgagtc gatatactgt aacaagctcg ggaaacataa  
121 gtacattttt tggagctatc ttttatgtt gcgcttttct ttgtctctt tgaatgagtt  
181 ttgaaatgaa ttgtctgtgc aaaaaaaaaa aa

//

660777-0542E+50